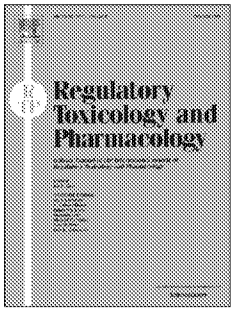
Accepted Manuscript



An evaluation of the USEPA Proposed Approaches for applying a biologically based dose-response model in a risk assessment for perchlorate in drinking water

Harvey H. Clewell, III, P. Robinan Gentry, C. Eric Hack, Tracy Greene, Rebecca A. Clewell

PII: S0273-2300(19)30036-4

DOI: https://doi.org/10.1016/j.yrtph.2019.01.028

Reference: YRTPH 4307

To appear in: *Regulatory Toxicology and Pharmacology*

Received Date: 19 October 2018

Revised Date: 18 January 2019

Accepted Date: 20 January 2019

Please cite this article as: Clewell III., , H.H., Gentry, P.R., Hack, C.E., Greene, T., Clewell, R.A., An evaluation of the USEPA Proposed Approaches for applying a biologically based dose-response model in a risk assessment for perchlorate in drinking water, *Regulatory Toxicology and Pharmacology* (2019), doi: https://doi.org/10.1016/j.yrtph.2019.01.028.

An Evaluation of the USEPA Proposed Approaches for Applying a Biologically Based Dose-Response Model in a Risk Assessment for Perchlorate in Drinking Water

Harvey H. Clewell III[1]*, P. Robinan Gentry[2], C. Eric Hack[3], Tracy Greene[2], Rebecca A. Clewell[4]

[1] Ramboll US Corporation, Research Triangle Park, North Carolina

[2] Ramboll US Corporation, Monroe, Louisiana

[3] ScitoVation, Research Triangle Park, North Carolina

[4] ToxStrategies, Research Triangle Park, North Carolina

*Corresponding Author: Harvey Clewell; hclewell@ramboll.com

ABSTRACT

The United States Environmental Protection Agency's (USEPA) 2017 report, "Draft Report: Proposed Approaches to Inform the Derivation of a Maximum Contaminant Level Goal for Perchlorate in Drinking Water", proposes novel approaches for deriving a Maximum Contaminant Level Goal (MCLG) for perchlorate using a biologically-based dose-response (BBDR) model. The USEPA (2017) BBDR model extends previously peer-reviewed perchlorate models to describe the relationship between perchlorate exposure and thyroid hormone levels during early pregnancy. Our evaluation focuses on two key elements of the USEPA (2017) report: the plausibility of BBDR model revisions to describe control of thyroid hormone production in early pregnancy and the basis for linking BBDR model results to neurodevelopmental outcomes.. While the USEPA (2017) BBDR model represents a valuable research tool, the lack of supporting data for many of the model assumptions and parameters calls into question the fitness of the extended BBDR model to support quantitative analyses for regulatory decisions on perchlorate in drinking water. Until more data can be developed to address uncertainties in the current BBDR model, USEPA should continue to rely on the RfD recommended by the NAS (USEPA 2005) when considering further regulatory action.

Keywords: perchlorate, risk assessment, MCLG, BBDR model

INTRODUCTION

From a regulatory perspective, the critical effect of concern from exposure to perchlorate is disruption of thyroid function and the potential for thyroid-hormone-related effects on neurodevelopment in gestation; these effects represent downstream events resulting from competitive inhibition of iodide uptake by the perchlorate ion (USEPA 2002). Based on an analysis of the mode of action for perchlorate, the United States Environmental Protection Agency (USEPA) (2002) determined that inhibition of thyroid iodide uptake could be used as an obligatory precursor for these critical effects in a harmonized cancer/noncancer risk assessment for perchlorate (Figure 1). This mode-of-action directed risk assessment approach was used in the derivation of the current Reference Dose (RfD) for perchlorate of 0.0007 mg/kg/day (USEPA 2005). Following the recommendations of the National Academy of Sciences National Research Council (NRC) (2005), the point of departure (POD) for this RfD was a reported No Observed Effect Level (NOEL): a non-statistically significant mean of 1.8% (standard error of the mean 8.3%) decline in radioactive iodine uptake (RAIU) in healthy adults following two weeks exposure to a daily perchlorate dose of 0.007 mg/kg/day (Greer et al. 2002). An intraspecies uncertainty factor of 10 was applied to protect the most sensitive population, the fetuses of pregnant women who might have hypothyroidism or iodide deficiency.

Subsequently, the USEPA Office of Drinking Water (2008) published an Interim Health Advisory Level for perchlorate of 15 µg/L, based on the USEPA (2005) RfD of 0.7 µg/kg/day, as recommended by the NRC (2005). Determination of this Interim Health Advisory Level considered Physiologically-Based Pharmacokinetic (PBPK) Modeling (Clewell et al. 2007) to estimate the potential effect of perchlorate on iodide uptake in several sensitive subgroups, including the pregnant woman and fetus, the lactating woman and neonate, and the young child. Despite widespread scientific acceptance of iodide inhibition as an obligatory precursor to downstream toxicity endpoints, there was remaining concern regarding the level of protection for the population perceived to have the greatest susceptibility – the fetuses of hypothyroid mothers.

Over the next several years, the focus of research on perchlorate shifted to the development of a biologically based dose-response (BBDR) model of the hypothalamic-pituitary-thyroid (HPT) axis that could be linked with the PBPK model of perchlorate and iodide to predict dose-dependent interactions

of perchlorate with iodine hormone homeostasis as a function of iodide intake in an effort to more quantitatively account for the effects of low dietary iodide intake and hypothyroidism in pregnant women on fetal development (McLanahan et al. 2008, 2009; Fisher et al. 2012; Lumen and George 2017a, 2017b; Lumen et al. 2013, 2015).

The USEPA Science Advisory Board (SAB) (2013) report on perchlorate in drinking water supported the utility of BBDR modeling to help characterize the potential for neurological effects from perchlorate exposure:

> "As perchlorate research continues, studies in animals may provide important insights into the neurobehavioral consequences of perchlorate exposure. A physiologically-based pharmacokinetic/pharmacodynamic framework is well suited to help place these findings in the context of human perchlorate exposure."

The USEPA SAB (2013) identified a number of areas for improvement or modification of the existing models. However, they also noted that "Models can always be improved, but the goal is to have a model that is fit for the intended purpose.", apparently cautioning against perpetual model refinement at the expense of implementation, echoing the concern of the renowned statistician, George E.P. Box, who famously used to say: "All models are wrong but some are useful" (Box 1976).

Recently, the USEPA's Office of Ground Water and Drinking Water (USEPA 2017) responded to the Science Advisory Board recommendations and proposed novel approaches to inform the derivation of a Maximum Contaminant Level Goal (MCLG) for perchlorate, including the use of BBDR modeling in their report entitled "Draft Report: Proposed Approaches to Inform the Derivation of a Maximum Contaminant Level Goal for Perchlorate in Drinking Water". This MCLG approach (USEPA 2017) includes revisions to a previously developed and peer reviewed BBDR model (McLanahan et al. 2008, 2009; Fisher et al. 2012; Lumen and George 2017a, 2017b; Lumen et al. 2013, 2015) that was extended to predict the relationship between perchlorate exposure and thyroid hormone levels in sensitive life stages. These revisions aim to address suggestions by the USEPA SAB (2013), including the following:

- Derivation of a perchlorate MCLG that addresses sensitive life stages through PBPK/PD modeling;

- Expansion of the modeling approach to account for thyroid hormone perturbations and potential adverse neurodevelopmental outcomes from perchlorate exposure;
- Utilization of a mode of action framework for developing the MCLG that links the steps in the proposed mechanism leading from perchlorate exposure through iodide uptake inhibition to thyroid hormone changes and finally neurodevelopmental impacts; and
- Extension of "the [BBDR] model expeditiously to…provide a key tool for linking early events with subsequent events as reported in the scientific and clinical literature on iodide deficiency, changes in thyroid hormone levels, and their relationship to neurodevelopmental outcomes during sensitive early life stages" (USEPA SAB 2013, p. 19).

Model revisions presented in the USEPA (2017) report include: incorporating a description of the physiology of early pregnancy, biological feedback control of hormone production via thyroid-stimulating hormone (TSH) and human chorionic gonadotropin (hCG), and a description of the response to lower levels of iodide nutrition. In addition, an attempt was made to calibrate the model's behavior for upper and lower percentiles of the population, in addition to the population median, for thyroid hormone production. The report also included an uncertainty analysis for key BBDR model parameters.

For the development of the MCLG, USEPA (2017) proposed a two-stage approach linking the revised BBDR model results ("Stage 1") with quantitative information on neurodevelopmental outcomes from epidemiological studies ("Stage 2"). Stage 1 describes the thyroidal hormone levels in women of childbearing age with low to adequate iodide intake. In this stage, the revised BBDR model is applied to predict the relationship between perchlorate exposure and changes in thyroid hormone levels in early pregnancy. Data for Stage 2 of the approach is provided from epidemiological studies evaluating maternal thyroid hormone levels in early pregnancy and the relationship between changes in these levels and the observation of neurodevelopmental outcomes. The USEPA (2017) report also described development of a novel population-based approach that uses the revised BBDR model to estimate changes in levels of selected thyroid hormones, specifically free tetraiodothyronine (fT4) and TSH, resulting from perchlorate exposure that may result in an increase in the prevalence of hypothyroxinemia in pregnant women. Hypothyroxinemia (low circulating concentrations of fT4) is

111 often associated with hypothyroidism (low concentrations of fT4 despite increased concentrations of
112 TSH).

113 The evaluation and (potential) application of the perchlorate BBDR model will serve as an important
114 precedent for future consideration of such models by the agency, as it is only the second such model
115 to be seriously evaluated by USEPA and subjected to external peer-review. The first BBDR model to
116 be considered, formaldehyde nasal carcinogenicity developed by Conolly and colleagues (2003, 2004),
117 has been under consideration by the agency for more than a decade. Interest in the use of BBDR
118 modeling in risk assessment peaked in the 1990s when the draft USEPA (2003) Cancer Guidelines
119 identified these models as the preferred option for performing a cancer dose-response. However,
120 since that time, work in this area has waned, possibly due to the perceived difficulty of gaining
121 regulatory acceptance. By their nature, BBDR models are descriptions of complex biological systems
122 that necessarily include significant uncertainty. The challenge going forward will be to develop
123 approaches for characterizing that uncertainty in a risk assessment context and ensuring that these
124 complex models are fit for their intended purpose. It is with this consideration in mind that we have
125 performed a focused evaluation of the proposed USEPA (2017) approaches.

126 Our critical review focused on two key areas of importance for determining whether the current BBDR
127 model is fit for the purpose of supporting regulatory decisions based on predicted effect of perchlorate
128 exposure on human fetal development:

129 1. Evaluation of USEPA (2017) model revisions to the peer reviewed BBDR models, including
130 extending the model to early pregnancy, incorporating biological feedback control of hormone
131 production via thyroid stimulating hormone (TSH) and human chorionic gonadotropin (hCG)
132 signaling, calibration of the model for thyroid hormone effects, and uncertainty analysis for
133 key parameters. This evaluation included comparison of model output to results from key
134 human studies identified in previous assessments (Greer et al. 2002, Braverman et al. 2006,
135 Téllez et al. (2005a, 2005b), as well as in the USEPA (2017) document (Steinmaus et al.
136 2016);
137 2. Evaluation of USEPA (2017) approaches for linking BBDR results to neurodevelopmental
138 outcomes and identification of published literature to develop the quantitative relationship
139 between thyroid hormone levels and neurodevelopmental outcomes; and

After describing the results of this evaluation, we present a comparison of the results from the USEPA (2017) approach with results from previous USEPA assessments, in order to put the uncertainties in the BBDR approach in perspective against the potential impact of the new approach on the existing regulatory guidelines for perchlorate USEPA (2005, 2008).

METHODS

Evaluating Stage 1 of USEPA MCLG approach: Stage 1 of USEPA's MCLG approach relies upon the application of the BBDR model to predict the effect of perchlorate on the thyroid hormone in pregnant women at different iodine nutrition levels, with the goal of predicting fT4 hormone reduction in pregnant women with low dietary iodide. To evaluate the utility of the proposed model to support such predictions, we independently ran the model and tested model predictions against data from multiple studies. These exercises attempted to both duplicate BBDR model results for datasets that were used by USEPA (2017) to calibrate the model and to test the ability of the BBDR model to predict the well-described precursor event inhibition of iodide uptake, which was successfully described with previous versions of the perchlorate PBPK models (Merrill et al. 2003; Clewell et al. 2007). These simulations included:

- Steinmaus et al. 2016 – cross-sectional epidemiological study evaluation of serum and urine in pregnant women in California: used in USEPA (2017) to evaluate BBDR model predictions of perchlorate effects on fT4 and TSH
- Greer et al. 2002 – 14-day controlled perchlorate dose study in male and female adults in the US: used in USEPA (2017) to estimate urinary clearance parameters in BBDR model
- Braverman et al. 2006 – 6-month controlled perchlorate dose study in male and female adults: not used in USEPA (2017)
- Téllez Téllez et al. 2005a, 2005b – longitudinal epidemiological study in pregnant and lactating women in Chile: used in USEPA (2017) to estimate urinary clearance parameters in BBDR model

In our efforts to produce these simulations, it was noted that instructions provided in the USEPA documentation for running the model for different scenarios, and documentation of the rationale for the model parameter values associated with them, are often inadequate and lack transparency; this deficiency is exacerbated by the number of permutations of parameter settings used in the scripts that

generate the results in the document. The complexity of the BBDR model makes it difficult to perform this evaluation, even though it has been conducted by experienced modelers.

Evaluating Stage 2 of USEPA MCLG approach: Stage 2 of USEPA's approach involved evaluating the published epidemiological literature to identify publications that would define quantitative relationships between thyroid hormone levels and neurodevelopmental effects. The USEPA approach was focused on the identification of studies that provided information on levels of fT4 in pregnant mothers during early gestation and the potential for changes in neurodevelopmental outcomes in their offspring. Through targeted literature searching and recommendations from the Science Advisory Board (SAB), a total of 55 studies were identified by USEPA to provide information on altered maternal thyroid hormone levels and offspring development. These studies were divided into three groups to facilitate evaluation:

- Group 1 – studies that may be able to quantitatively describe a relationship between incremental alterations in maternal thyroid hormone levels and alternations in offspring development;
- Group 2 – studies that do not have data from which to derive a quantitative relationship between maternal hormones and offspring neurodevelopment, but instead present only a categorical analysis with thyroid hormones below and above a defined cut point and adverse neurodevelopmental outcomes; and
- Group 3 – studies that present an analysis that is not directly compatible with BBDR output.

Of the 55 studies, 15 were identified as Group 1, 14 were identified as Group 2, 26 were identified as Group 3. The 15 Group 1 studies were then evaluated further and only 5 were deemed useful by the USEPA for further quantitative analysis to attempt to connect alterations in thyroid hormone levels to alterations in neurodevelopment. in our evaluation, we performed a critical review of the USEPA Stage 2 approach and the study summaries provided in USEPA (2017), considering the most recent recommendations from the National Research Council (NRC 2014) on systematic review of the literature and evidence integration.

RESULTS

Evaluation of the Perchlorate BBDR Model for Early Pregnancy

The draft MCLG approach (USEPA 2017) is based on a hypothesized mode of action (Figure 1) for neurodevelopmental outcomes resulting from development of hypothyroxinemia from perchlorate-induced inhibition of iodide uptake in the thyroid. As noted in USEPA (2017):

> "Thyroid hormones are essential for the development and differentiation of the developing brain. The brain and spinal cord begin development in the first half of the first trimester. fT4 passes through the blood-brain barrier via multiple, specific transporter proteins. Next, T4 is converted to T3 by the developing glial cells and then transported to neurons. T3 then interacts with nuclear receptors to tightly regulate gene expression so that neurogenesis, synaptogenesis, neuronal migration, cell differentiation, and myelination are developmentally appropriate. Deficiencies in thyroid hormones through iodine deficiency, congenital hypothyroidism, or maternal hypothyroidism/hypothyroxinemia can result in neurological impairments and intellectual deficits (Morreale de Escobar, Obregón, & Escobar del Ray 2000)."

As recommended by the USEPA SAB (2013), the USEPA extended a published BBDR model for perchlorate induced hypothyroxinemia in late gestation (Lumen et al. 2013; Lumen and George 2017a, 2017b) to address the sensitive population of concern for exposure to perchlorate: the fetuses of hypothyroxinemic women during early pregnancy (Figure 2). These concerns were motivated by new studies (Steinmaus et al. 2016), suggesting an association between perchlorate exposure and decreased levels of free thyroxine (fT4) in pregnant women. Because the fetus is entirely dependent on maternal thyroid hormones for neurodevelopment in early gestation (Clewell et al. 2007; Howdeshell 2002), the endpoint of interest was defined as reduction in maternal fT4 in early pregnancy and the perchlorate BBDR models were extended to describe hormone homeostasis during gestation. Expansion of the original models of perchlorate and iodide (Clewell et al. 2007) to predict the impact of perchlorate exposure on fT4 during early pregnancy, however, is complicated by the significant variability in the levels of fT4 in the general population and the challenges in measuring fT4, as well as the dynamics of changing hormones through the course of gestation and the uncertainty in identifying the level of alteration that may lead to hypothyroidism and fetal effects.

224

225 According to the "American Thyroid Association Task force on Thyroid Disease During Pregnancy and
226 Postpartum", isolated hypothyroxinemia is defined as a normal maternal TSH concentration in
227 conjunction with fT4 concentrations in the lower 5th or 10th percentile of the reference range
228 (Stagnaro-Green et al. 2011). USEPA (2017) has also focused on selected percentiles of the reference
229 range; however, reference ranges can vary from population to population according to the *2017*
230 *Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease*
231 *During Pregnancy and Postpartum* (Alexander et al. 2017). Even within US populations and across
232 ethnic groups, the 2.5th percentile can vary by up to 2 pmol/L or approximately 20% (9.3-11.4
233 pmol/L as reported by Alexander et al. 2017).

234 The variation in fT4 reported in the published literature during early pregnancy is provided in USEPA
235 (2017), Appendix A, Figure A-33 and reproduced in Figure 3. The levels of fT4 during early
236 pregnancy, based on the studies identified by USEPA (2017), appear to range from approximately 13-
237 17 pmol/L. This range is consistent with the range of baseline fT4 means reported in the Greer et al.
238 (2002) study of approximately 1.1 – 1.3 ng/dL (14 - 17 pmol/L). However, the 50th percentile BBDR
239 model predictions at zero dose perchlorate and 170 µg/day iodine intake are approximately 10 pmol/L
240 at gestation weeks 12, 13, and 16, considerably below these reported values.

241

242 Measuring fT4 in the presence of high concentrations of bound T4 is challenging, especially in
243 conditions where binding proteins are altered such as during pregnancy (Alexander et al. 2017).
244 Measurement techniques are prone to inaccuracy during pregnancy due to disruption of the original
245 equilibrium. The 95% fT4 reference intervals decrease gradually with advancing gestational age: from
246 1.08– 1.82 ng/dL (approximately 13.9 – 23.5 pmol/L) in week 14 to 0.86–1.53 ng/dL (approximately
247 11.1 – 19.8 pmol/L) in week 20 (Alexander et al. 2017).

248 Extending the thyroid BBDR model to address early gestation is particularly challenging due to
249 the complex interaction between thyroid homeostasis and gestational development.
250 Considering the addition of TSH feedback dynamics, and an adjustment factor to match
251 specific population percentiles, there is reason for concern regarding the uncertainty of the

revised model predictions under low iodide intake conditions. Some of these concerns are highlighted below:

Description of hCG dynamics: Human chorionic gonadotropin (hCG) levels rise in early pregnancy and this in turn increases both sodium-iodide symporter (NIS) uptake activity and T4 production. hCG is structurally similar to TSH and, like TSH, increases thyroidal iodide uptake and thyroid hormone synthesis by binding to the thyroid-stimulating hormone receptor (TSHR) (Hoermann et al. 1994). In the model, hCG levels are calculated as a function of gestational age, using an equation for the parameter HCGREG (Figure 4, purple curve), and these changing levels are used to increase the rate of T3 and T4 production as a function of the hCG concentration:

$$HCGreg = 1 + 0.00354*hCG$$

The variation of hCG over the duration of gestation is based on direct measurements of hCG in pregnant women (Korevaar et al. 2015). However, the concurrent increase in thyroidal iodine uptake is described in the model based on an empirical relationship between gestational age in weeks (GW) and radioactive iodide uptake, using an equation for the parameter VCHNG (Figure 4, green curve):

$$VCHNG(GW) = 1 + 0.076 \cdot GW - 0.0025 \cdot GW^2$$

Thus the model does not correctly attribute the gestational control of NIS uptake to hCG, when in fact both uptake and hormone synthesis respond to the same changes in hCG (Pesce and Kopp 2014). By using different equations for the time-dependence of hCG-stimulated uptake and hormone production (Figure 4) the model decouples processes that are fundamentally linked by their biology. Figure 4 depicts the time-course of the parameters controlling changes in iodide uptake (VCHNG) and hCG hormone levels (HCGreg) over the course of gestation. While the biology indicates a proportional relationship between the two parameters, the equations used in the model are not parallel. Elucidating the impact of this decoupling is challenging, and is beyond the scope of this review, since it would have to be investigated at a large number of time-points throughout pregnancy and under different conditions of iodine intake, but the disparity between the model description and the underlying biology justifies caution regarding its predictions of T4 and TSH at different gestational ages, as these parameters govern hormone production and

release. We address the impact of the discrepancy between the time-courses for HCGREG and VCHNG in a later section.

Damping of TSH response: The USEPA (2017) BBDR model includes a parameter, pTSH (power to which the ratio of current TSH to the TSH set-point is raised), that reduces the response of the thyroid to increases in TSH:

$$TSHreg = (TSH/TSH_0)^{pTSH}$$

Using this equation, a pTSH exponent of 0 would represent no control of thyroid function by TSH and an exponent of 1 would represent a linear response of thyroid function to changes in TSH. In their calculations of the effect of perchlorate on the prevalence of hypothyroxinemic pregnant women, the USEPA (2017) use a pTSH exponent of 0.398, which results in a response to TSH that is substantially less than linear, an assumption that is inconsistent with the fundamental biological relationship between TSH and thyroid hormones (production and release of T3 and T4), effectively decoupling a relationship that has been well established in the medical, pharmacological and toxicological communities. USEPA (2017) describes the rationale for this parameter: "The NHANES data do not show a clear correlation between TSH and fT4, so within that data set they vary independently. One could assume, therefore, that individuals with an average fT4 and high TSH have that combination because their thyroid has a weak response to TSH, and vice-versa." To address this concern, USEPA (2017) estimated a lower and upper bound for pTSH as (median TSH)/(97.5th percentile TSH) = 0.398 and (median TSH)/(2.5th percentile TSH) = 3.09, respectively, with a median value of pTSH = 1. Thus, this parameter is used to attempt to represent disease states where the individual's thyroid is either exquisitely sensitive or insensitive to TSH stimulation. At lower values, this parameter reduces the impact of TSH on the Vmax for thyroid iodide uptake as well as the rate constants for T4 and T3 production in the thyroid. However, the USEPA (2017) also states that: "The coefficient, pTSH, is included…to allow for tuning of the strength of the TSH feedback, but in practice model simulations versus data appear quite adequate with pTSH=1." Concerns about this parameter are two-fold. First, the complexity of the model and various runtime scripts makes it nearly impossible to determine the use of this

parameter during some of the model assessment and risk assessment simulations presented in USEPA (2017). Second, using point estimate population level data to define the quantitative temporal relationship between two fundamentally linked processes at the individual level is scientifically inappropriate. To understand the biological feedback within a single individual (i.e. to determine the relationship of TSH to T3/T4 and Vmax for a hypothyroid or hyperthyroid individual), matched samples would be needed for TSH, T3 and T4. This information – to our knowledge – is not available from NHANES. Thus, the epidemiological point estimate data are being used well beyond its domain of applicability to predict the quantitative outcome of disease states.

Calibration of hormone production rates: The model uses a baseline first-order constant calibrated to NHANES 2007-2012 median, 10th, or 90th percentile non-pregnant data (fT4, fT3, T4 and T3 concentrations). The model parameter for the rate of production of T4 (KProdT4F) for the median NHANES calibration used in USEPA (2017) is 6.25*10-7 /hr/$kg^{0.75}$ (their Table A-2), which is 4-fold lower than the value of 2.45 x 10-6 estimated for the published model (Lumen et al. 2013), which was based on the data of Nicoloff et al. (1972). However, the use of a T4 production rate that is lower than the published value is not adequately justified, given the importance of this parameter, which has a direct impact on predictions of fT4 changes, the intended application of the model. This baseline value is then scaled in pregnancy through GW 16 (peak occurring ~ GW 9) based upon placental hCG increase over this time, according to the linear relationship from Glinoer (1997): hCGreg = 1 + 0.00354 x hCG.

Affinity of NIS Iodine uptake: The model uses a Km for perchlorate binding to the NIS (KmNIS_P) that is 3-fold lower than the value estimated by Lumen et al. (2013) (i.e. a 3-fold higher affinity). Specifically, the new Km represents the 2.5th percentile lower confidence limit of the population median based upon the USEPA (2017) reanalysis of Greer et al. (2002). The median value (50th percentile = 0.73 µM) is similar to that obtained from a re-analysis of *in vitro* binding data, 0.59 µM (Schlosser 2016); the use of a value of KmNIS_P = 0.489 µM makes perchlorate 3 times more effective at competitive inhibition of NIS compared to the model of Lumen et al. (2013). This revision to the Km in USEPA (2017) necessitated revisions to the Vmax (VmaxNISF_thy_P) and urinary excretion parameters (CLFUP) (Table 1 of USEPA 2017), further affecting the model's

sensitivity to changes in perchlorate dose, particularly under conditions of low iodide. Thus, The USEPA (2017) BBDR model predicts much greater effects of perchlorate on iodide uptake than any previous version of the model, without justification for re-estimating these parameters rather than using the published values.

Assumptions regarding thyroidal iodide storage: Plots of NHANES 2007-2012 data for non-pregnant women demonstrated little relationship between iodine intake and fT4, even at iodide intake levels below 75 µg/day (Figure A-54 of USEPA 2017; reproduced in Figure 5a). USEPA (2017) used data on the relationship between thyroidal iodide stores (mg) and iodine intake from Delange (2000), which assumes depletion of fT4 at iodide intake levels below 100 µg/day. As is clear from Figure 5b, this assumed model behavior at concentrations below 100 µg/day, which drives model predictions at low intakes, is inconsistent with the NHANES data and could result in overprediction of fT4 responses at moderately low intakes of iodide, including the ranges simulated in the USEPA report. This possibility was investigated in this evaluation and the results are discussed in the next section.

Evaluation of BBDR Model Behavior

Comparison to the Steinmaus et al. 2016 Results

In Appendix B of USEPA (2017), a comparison of the predicted changes in both fT4 and TSH from the BBDR model were compared to the results reported by Steinmaus et al. (2016). The Steinmaus et al. (2016) study was conducted to evaluate the potential for perchlorate exposure to impact thyroid hormone levels in pregnant women (any trimester) in San Diego. They reported an effect of perchlorate on fT4 levels to be similar among women with both low iodine (<100 µg/day) and normal (100-300 µg/day), with a greater effect of perchlorate observed among pregnant women in the high iodine intake group (>300 µg/day). They further noted that this result is in contrast to some previous results from NHANES (Blount et al. 2006) and may be due to the overall iodine sufficiency in the studied population or the fairly long time between urine iodine and serum thyroid hormone sample collection (about 9 weeks).

The comparison of the predicted fT4 changes from the BBDR model and the Steinmaus et al. (2016) results associated with changes in perchlorate dose are reported in Figure B-1 of Appendix B of USEPA (2017) and reproduced in Figure 6. This comparison, which we were able to reproduce using the

366 USEPA (2017) BBDR model, clearly highlights the differences between the model predictions and the
367 published human data. The USEPA (2017) BBDR model simulations with normal iodine intake (170
368 µg/day) demonstrate no change in fT4, which is consistent with other studies in which no impact on
369 fT4 has been observed at doses up to 7 µg/kg/day perchlorate (Greer et al. 2002; Braverman et al.
370 2006). The USEPA (2017) BBDR model greatly under-predicts the changes in fT4, even in the
371 scenario with low dietary iodine intake (75 µg/day), in comparison to the changes reported by
372 Steinmaus et al. (2016). This discrepancy raises concerns about the ability of the USEPA (2017)
373 BBDR model to predict changes in fT4 associated with chronic perchlorate exposure during pregnancy.

374

375 *Greer et al. 2002 – 14 day human controlled perchlorate dosing study*

376 The Greer et al. (2002) study was conducted to establish the dose-response in humans for perchlorate
377 inhibition of thyroidal iodide uptake and any short-term effects on thyroid hormones following
378 exposure for male and female volunteers to perchlorate in drinking water at doses of 7, 20, 100 or
379 500 µg/kg/day for 14 days. The results of this study have previously been relied upon by the USEPA
380 (2005) to derive a reference dose (RfD) and to determine health reference levels (HRLs). The results
381 of this study indicate a decrease in iodide uptake following exposure to a dose of 20 µg/kg/day, but no
382 effect on hormone levels, including fT4 and TSH, at the highest dose tested. A No Observed Effect
383 Level (NOEL) of 7 µg/kg/day was determined based on these results, and an RfD of 0.7 µg/kg/day
384 was adopted, based on NAS recommendations, with the application of an uncertainty factor of 10 for
385 intraspecies variability or sensitive subpopulations.

386 Consistent with the results of the study, our simulations of the adult exposures reported in Greer et al.
387 (2002) with the BBDR model (Table 1) indicated no significant change in fT4 at doses up to 500
388 µg/kg/day. However, predicted concentrations of fT4 are lower than those measured by Greer et al.
389 (2002). The model simulation reported in Table 1 was run with an iodine intake of 90 µg/day, as this
390 was the value USEPA (2017) used in the Greer_test.m script provided with the BBDR model code.
391 However, 90 µg/day is not consistent with the 170 µg/day value USEPA (2017) reports as
392 representing a sufficient intake and USEPA's (2017) documentation does not indicate why a lower
393 value was used for the individuals in the Greer study. Simulation of iodide uptake inhibition (RAIU)
394 appears to over-predict the reduction in uptake compared to measured values, though the qualitative

increasing trend of inhibition with dose behaves appropriately. This discrepancy may result from the low iodine intake chosen by USEPA (2017), or a number of other decisions made in the model revisions, including the reduced Km parameter value. It is unclear why the parameters governing iodide inhibition were altered from previous models that successfully predicted inhibition of iodide in human subjects (Clewell et al. 2007; Merrill et al. 2003; Lumen et al. 2013). Given that iodide inhibition is the obligatory precursor to all downstream effects in the USEPA's proposed mode of action for perchlorate, it would be expected that any changes to the model that lead to reduced accuracy in the prediction of iodide inhibition would be accompanied by substantial support. However, no such support is provided in USEPA (2017) for the changes in the key parameters and the resulting effect on iodide inhibition predictions.

Table 1. Simulation of the Greer et al. (2002) Perchlorate Study

Dose	RAIU (%)		fT4 (pM)	
(µg/kg/d)	Simulated	Measured	Simulated	Measured
0	100	100	10.33	-
7	89	98.2	10.33	-
20	74	83.6	10.32	16.09
100	37	55.3	10.31	15.26
500	11	32.9	10.30	15.44

Braverman et al. 2006 – 6 month human controlled perchlorate dosing study

The Braverman et al. (2006) study was conducted to determine whether prolonged exposure (6 months) of adults to low levels of perchlorate (0.5, 1.0 or 3.0 mg/day) would perturb thyroid function. The study included a small number of individuals (n=13); however, iodine levels were comparable with those of the general population. The authors noted the limitations of the small sample size, but concluded that the results suggested that healthy, euthyroid individuals, with normal levels of iodine intake, can tolerate chronic exposure to perchlorate at doses of up to 3 mg/day (approximately 40 µg/kg/day) without any effects on thyroid function, including inhibition of iodine uptake.

The Braverman et al. (2006) study was simulated as part of the current evaluation using the BBDR model and predicted T3 and TSH levels were compared to the reported measurements (Table 2). fT4

was not compared because it was not clear how to convert the T4 index reported in the study to a concentration and vice versa. As with the Greer et al. (2002) simulation, 90 µg/day was used for iodine intake. Baseline T3 and TSH are similar to the measured values. But, as was seen with fT4, the model fails to predict the observed changes in hormone levels in the adult subjects.

Table 2. Simulation of Braverman et al. (2006) perchlorate study.

Dose	T3 (nM)		TSH (mIU/L)	
(µg/kg/d)	Simulated	Measured	Simulated	Measured
0	2.63	2.49	1.51	1.20
7	2.63	2.51	1.52	1.60
43	2.62	1.77	1.53	2.60

Téllez Téllez et al. 2005a, 2005b – Chilean epidemiological study in pregnant women

Téllez Téllez et al. (2005a, 2005b) reports the results of a longitudinal epidemiological study among pregnant women from three cities in Chile exposed to concentrations of perchlorate as high as 114 µg/L in the public drinking water. The focus of the study was to evaluate maternal thyroid function during pregnancy, neonatal thyroid function and developmental status at birth, and breast milk iodine and perchlorate levels during lactation. The National Academy of Sciences (2005) has reviewed this study in the context of health implications for perchlorate ingestion and concluded this study should be considered in the evaluation of the US experience with perchlorate in drinking water. The total iodine nutrition among this cohort was also noted to be similar to that of US pregnant women (Téllez Téllez et al. 2005a); therefore, this study should be a key consideration in evaluating the relationship between perchlorate exposure, changes in fT4 in pregnant women and developmental status; however, it was not considered in Stage 2 of the USEPA (2017) assessment because it pre-dated the cutoff used by USEPA in their review (2010).

Results from this study indicated no effect on thyroid levels in early pregnancy, late pregnancy, or neonates at birth related to perchlorate in drinking water at concentrations up to 114 µg/L. Given these findings, this study provides a reasonable dataset for validating the impact of high perchlorate exposure concentrations in drinking water on potential changes in fT4 or TSH.

438 We also ran the (USEPA 2017) BBDR model to simulate the Téllez Téllez et al. (2005a, 2005b)
439 drinking water study (Table 3). The BBDR model predictions of fT4 for GW 13-16 are consistent with
440 the negative results of the study, though the predicted concentrations are lower than those observed.
441 This is not a strong validation of the model given the weak trend of changes in hormone levels seen in
442 comparisons to other studies.

Table 3. Simulation of the Téllez Téllez **et al. 2005a, 2005b study of pregnant women exposed to perchlorate via drinking water.**

Dose	**fT4 (pM)**	
(µg/kg/d)	**Simulated**	**Measured**
0.01	9.74	12.5
0.08	9.73	12.2
2	9.69	12.7

443 *Summary: Evaluation of Model Behavior*

444 Our simulations of the Greer et al. (2002) and Braverman et al. (2006) studies with the BBDR model
445 indicate that thyroid hormone levels are relatively insensitive to inhibition of thyroid iodine uptake by
446 perchlorate exposures as high as 7 µg/kg/day. Moreover, our simulations of the Téllez Téllez et al.
447 (2005a, 2005b) study with the BBDR model do not predict an effect on fT4 from exposures to
448 perchlorate at up to 2 µg/kg/d, consistent with the fact that the exposures were demonstrated to be
449 without effect to pregnant women in the study. However, the USEPA (2017) BBDR modeling analysis
450 (Table 4, taken from USEPA 2017)predicted population-level changes in fT4 deficiency during the first
451 trimester at perchlorate exposures nearly an order of magnitude lower (0.3 µg/kg/d) . This
452 discrepancy suggests that the metric used in the USEPA (2017) approach to assess population-level
453 effects of perchlorate, i.e., a 1% or 5% increase the proportion of thyroxinemic mothers in early
454 pregnancy assuming that all individuals have a low (75 µg/day) iodine intake and an inadequate TSH
455 response (pTSH = 0.398 vs. 1), may be overly conservative.

456

Table 4. Summary of Results for the Amount of Perchlorate Needed to Increase the Proportion of Hypothyroxinemic, Low Iodine Individuals by a Defined Percentage (with hypothyroxinemia defined as fT4< 10th Percentile) (USEPA 2017)

Gestational Week	fT4 (pmol/L) at the Hypothyroxinemic Cut Point (i.e. 10th Percentile of 170 µg/day Iodine Intake Group) (Column 1)	Corresponding Percentile in 75 µg/day Iodine Intake Group (Column 2)[a]	Perchlorate Dose (µg/kg/day) Associated with a 1 Percent Increase in Proportion Hypothyroxinemic (Column 3)[a]	Perchlorate Dose (µg/kg/day) Associated with a 5 Percent Increase in Proportion Hypothyroxinemic (Column 4)[a]
12	8.80	48.4	0.4	2.2
13	8.78	47.9	0.4	2.2
16	8.63	52.6	0.3	2.1

[a] Results based on central effect estimates, pTSH in BBDR model set to 0.398

457

458 ***Evaluation of the effect of model assumptions on predicted PODs***

459 In order to assess the potential quantitative impact of some of the uncertainties in the BBDR
460 model, we compared model predictions of percent change in fT4 and TSH for a range of
461 perchlorate concentrations using two alternative parameterizations: (1) the parameterization
462 used by the USEPA (2017) to generate their Table 3, and (2) replacing the equation for
463 HCGREG with the equation for VCHNG (in order to provide an appropriately coupled response
464 to hCG stimulation of thyroidal iodine uptake and thyroid hormone production), and also
465 setting pTSH = 1 (the nominal value, as opposed to the lower-bound value of 0.398 used by
466 the USEPA). The simulations (Table 5) were performed with the model calibrated to either the
467 median population thyroid hormone levels (using the script medset.r) or a low (thyroxinemic)
468 population defined as fT4 < 10th percentile (using the script lowset.r). When predicting the
469 effect of perchlorate exposure on fT4 for the median population there is not a significant
470 difference between the USEPA results and the alternative parameterization; however, the
471 USEPA model parameterization results in more than a factor of 2 greater sensitivity of TSH
472 levels to perchlorate compared to the alternative parameterization. This difference is primarily

473 due to the change in pTSH. On the other hand, when predicting the effect of perchlorate
474 exposure on hypothyroxemic individuals, both fT4 and TSH responses to perchlorate exposure
475 are significantly lower using the alternative parameterization. Thus, the parameters that were
476 altered in the recent revision of the model (VCHNG, HCGreg, pTSH, KmNiS_p) increase the
477 predicted effect on thyroid hormone levels compared to the expected response with the well-
478 validated precursor event of iodide inhibition. The sensitivity of the prediction to changes in
479 these parameters, and the disconnect between the prediction of iodide inhibition and thyroid
480 hormone levels, calls for better justification – and evaluation – of the given parameter values.

Table 5. Predicted fT4 and TSH Concentrations at Various Doses of Perchlorate for 75 µg/day Iodine Intake

Perchlorate Dose (µg/kg/day)	fT4 (pmol/L) (% Change from 0 Dose)				TSH (mIU/L) (% Change from 0 Dose)			
		USEPA[0]	VCHNG + pTSH[1]	VCHNG + pTSH[1]		USEPA[0]	VCHNG + pTSH[1]	VCHNG + pTSH[1]
	Population	Median	Median	Low	Population	Median	Median	Low
0	Absolute	8.6	9.9	7.5	Absolute	2.2	1.5	3.0
1		-0.74	-0.8	-0.31		3.3	1.4	1.9
2		-1.5	-1.6	-0.61		6.6	2.7	3.8
3	Percent	-2.1	-2.3	-0.9	Percent	10	4.1	5.7
4	Change	-2.8	-2.9	-1.2	Change	14	5.5	5.7
5		-3.4	-3.5	-1.5		17	6.9	7.7
10		-6.2	-6.2	-2.8		36	14	19

[0] Results using pTSH = 0.398

[1] Results using HCGREG replaced with VCHNG, and pTSH=1

481 **Review of Literature Linking BBDR Results to Neurodevelopment Outcomes**

482 Chapter 5 of USEPA (2017) focuses on the SAB's recommendation to "Identify literature and conduct
483 analyses to support the model outputs for the downstream steps" from the BBDR's predicted changes
484 in thyroid hormones following exposure to perchlorate. Specifically, Chapter 5 was developed to
485 present the process USEPA (2017) used to identify literature to support the draft approach for
486 derivation of the MCLG for perchlorate. USEPA (2017) states, "Based on the recommendations of

487 previous peer review panels, USEPA assumed that changes in thyroid hormone levels would be
488 expected to lead to neurodevelopmental outcomes", and because of this assumption, a complete
489 systematic review of the body of literature on this topic was not performed. Instead, a "focused
490 review of the published literature" was conducted.

491 The approach is inconsistent with recent recommendations from the National Research Council (NRC
492 2014) regarding systematic review and evidence integration. These recommendations are currently
493 being incorporated into the USEPA's Integrated Risk Information System (IRIS) process and USEPA
494 has recently released scoping and problem formulation materials for several new Integrated Risk
495 Information System (IRIS) assessments, including ethylbenzene (USEPA 2014a), and naphthalene
496 (USEPA 2014b). The approach applied in these assessments is intended to follow recommendations
497 provided by the National Research Council (NRC 2013). While development of MCLGs are not part of
498 the IRIS process, the application of systematic review principles in the identification of studies to
499 define the relationship between fT4 and neurodevelopmental effects, is needed. The application of
500 these principles would not only assist in defining the highest quality studies to address a specific
501 research question, they also provide a way to integrate all of the available evidence for the specific
502 research questions raised by the SAB. Systematic reviews include the formulation of a specific
503 question to be addressed and developing a protocol that specifies the methods that will be used to
504 address the question. While a broad research question can lead to a large systematic review, if the
505 research question is limited, such as in the case of perchlorate, then the systematic review becomes
506 more focused.

507 For the USEPA (2017) draft MCLG approach, a systematic review question could have been easily
508 developed based on the SAB recommendation (i.e. "Identify literature and conduct analyses to support
509 the model outputs for the downstream steps") and the protocol would simply be focused on the
510 methods for conducting the systematic review to address this very focused systematic review question
511 in a transparent manner. Transparency being defined by USEPA as "sufficient information will be
512 available to understand the scientific rational behind decisions, as well as, reproduce methods used to
513 identify and evaluate data". However, in the case of the literature identified for consideration in the
514 draft MCLG approach for perchlorate, a well-defined protocol for all steps of the process has not been
515 developed and therefore is inconsistent with the recommendations of the NRC (2013):

> *"A priori decisions and a predefined protocol are critical during the systematic review process (Berlin and Colditz 1999; Dickersin 2002); the protocol should describe the following steps: the research question, the search strategy and data sources, the study inclusion and exclusion criteria, the data to be abstracted and derived from the original studies (such as sample size, exposure and outcome assessment methods, and confounders evaluated), the criteria and methods for pooling effect estimates and measures of variability among studies. Systematic reviews and meta-analyses need to be replicable; other investigators following the same steps should be able to identify the same articles, abstract the same data, and reach similar conclusions."*

At each step of the process for identifying studies for use in the development of the MCLG approach for perchlorate, a detailed set of criteria is needed. For example, if decisions are made to include or exclude any studies, there should be very detailed criteria indicating why studies were included or excluded and it should be specified prior to the initiation of the literature searching process. The criteria for each step should be described in such a way that an independent reviewer could use it to replicate the results of the literature search and review; however, there are several areas in the USEPA (2017) draft MCLG approach for perchlorate where this level of detail is lacking, making it difficult for an independent reviewer to replicate the results.

Systematic Review Research Questions

An overall hypothesis or systematic review research question should be developed that is based on the SAB recommendation to clarify the focus of the review and the linkage between altered maternal fT4 (as predicted by the BBDR model) and the potential for adverse neurodevelopmental effects in offspring. Some additional explanation as to how USEPA arrived at the specific neurodevelopmental outcomes of concern should be provided.

Searching the Published Literature

While the literature search key words are presented in the USEPA (2017) report, there is a lack of explanation as to the reasoning behind the focus on the outcome of concern. The research question should be used to develop the literature search. The major points used or considered in developing

544 the literature search strategy should be presented. In addition, there should be a detailed explanation
545 of the criteria used to screen the literature search results. Furthermore, USEPA (2017) does not
546 report the details of the literature search results. For each search string reported in Table 9 of the
547 USEPA (2017) report, a total number of citations identified should be reported. In addition, the
548 criteria used to screen the original search results should be clearly reported in the document.
549 Essentially, each step of the literature search and review should be reported in such a way that any
550 independent party could easily reproduce the results reported in Chapter 5 of USEPA (2017). The lack
551 of this type of information does not allow the reader to determine if any key studies may have been
552 removed from consideration.

553 *Literature Screening Approach and Selection of Key Studies*

554 USEPA (2017) states that a 3 step approach was used to identify studies for consideration in the
555 development of the approach for derivation of the MCLG for perchlorate. The approaches utilized by
556 USEPA (2017) to identify the epidemiological studies for this evaluation were strictly focused on the
557 appropriateness of the quantitative data for consideration in combination with the output of the BBDR
558 model. Group 2 (studies with categorical analyses only) and Group 3 (studies with analyses not
559 directly compatible with BBDR output) studies were apparently eliminated from consideration in the
560 assessment. While not directly compatible with BBDR modeling output, it is possible that these
561 studies may provide information important in understanding the potential relationship between
562 changes in thyroid hormones and the potential for neurodevelopmental effects, as well as potential
563 key confounders.

564 While 15 studies were identified in Group 1, only 5 of these were determined by USEPA to include
565 analyses that could be used to connect the results of the BBDR model to incremental changes in
566 adverse neurodevelopmental effects. A clearly defined set of inclusion and exclusion criteria should be
567 provided to clearly convey to the reader why the other 40 studies in Groups 1, 2, and 3 were not
568 considered. In addition, studies that provide no evidence of an inverse relationship between
569 perchlorate exposure and serum thyroid function (e.g. Ghassabian et al. 2014; Modesto et al. 2015;
570 Moleti et al. 2016; Noten et al. 2015) should also be considered to not only understand why these
571 results are in contrast to the potential research question, but also that the overall weight of evidence
572 can be determined. It is possible that the majority of studies provide evidence that critical factors that

573 are not reported in some of the available studies may explain the reported changes in serum thyroid
574 function.

575 *Assessment of Study Quality and Risk of Bias*

576 According to recent recommendations from the National Research Council (NRC 2014), the National
577 Toxicology Program's (NTP) Office of Health Assessment and Translation (OHAT) method for the
578 assessment of study quality and risk of bias of the literature (NTP 2015) is one method that should be
579 considered for qualitative and quantitative assessments. "An assessment of study quality evaluates
580 the extent to which the researchers conducted their research to the highest possible standards and
581 how a study is reported. Risk of bias is related to the internal validity of a study and reflects study-
582 design characteristics that can introduce a systematic error (or deviation from the true effect) that
583 might affect the magnitude and even the direction of the apparent effect" (NRC 2014). Each study
584 meeting inclusion criteria in Group 1, 2, and 3, should be evaluated against a predetermined set of
585 study quality and risk of bias criteria and the results of this evaluation should be presented in the
586 perchlorate MCLG approach report.

587 Uncertainties Critical to Characterizing Changes in Thyroid Hormone Levels in Pregnant Women
588 Associated with Neurodevelopmental Changes in Offspring

589 The draft MCLG approach presented in USEPA (2017) to predict doses of perchlorate that would result
590 in per unit changes in neurodevelopmental measures, is, as noted by USEPA (2017), "…dependent
591 upon predictions from the BBDR model, the derivation of the distribution of fT4, and the evaluations of
592 the relationship between fT4 and neurodevelopment. Each of these steps has inherent uncertainties
593 associated with it."

594 A major source of uncertainty is related to the five studies in Group 1 with data that could be used to
595 quantitatively describe the relationship between thyroid hormone levels in early pregnancy and
596 changes in neurodevelopment (Pop et al. 1999, 2003; Finken et al. 2013; Korevaar et al. 2016;
597 Vermiglio et al. 2004). None of these five studies relied upon data from US populations or have been
598 demonstrated to have iodine intake similar to US populations. Yet according to the American Thyroid
599 Association (Alexander et al. 2017), the reference range of both TSH and fT4 in pregnant women
600 varies depending upon ethnicity. While two studies in Group 1 focused on population groups within

601 the United States, neither were considered for the model because T4 and not fT4 was measured in the
602 pregnant females (Oken et al. 2009) and the relationship between fT4 and neurodevelopment was
603 evaluated in late pregnancy and did not reach statistical significance (Chevrier et al. 2011). USEPA
604 (2017) (Section 6.5.1) states "there is no reason to believe that the impact of fT4 on
605 neurodevelopment would differ by country, unless there is a substantial difference in iodine intake".
606 While USEPA (2017) does make an effort to evaluate changes in iodine intake in women from various
607 populations, including the US, there are not substantial data reported in the peer-reviewed literature
608 to validate the conclusions that the impact of fT4 on neurodevelopment would differ by population or
609 uncertainty in iodine intake levels would have an impact on the derivation of the MCLG. This is
610 inconsistent with data from the American Thyroid Association (Alexander et al. 2017) that suggest
611 variability in the distribution of thyroid hormone levels across populations and even within ethnicities
612 within a single population.

613 USEPA (2017) also notes that all five studies used for quantitative analysis relied on a one-time fT4
614 level during pregnancy (Section 6.5.5). The influence of changes in maternal fT4 on fetal brain
615 development is likely greatest during early pregnancy. The variability in maternal fT4 levels during
616 pregnancy and the lack of measurement of fT4 at time points throughout pregnancy in the studies
617 provides a substantial data gap and lack of information needed to validate some of the assumptions
618 relied upon in the development of the BBDR current model as well as the resulting predictions of the
619 model. As stated in USEPA (2017),

620 *"Circulating T3 and T4 levels in an individual are maintained within a narrow range by*
621 *a negative feedback loop with TSH from the pituitary and TRH from the hypothalamus*
622 *that operates around a "set-point." This set-point is different from individual to*
623 *individual, which generates a population variance in blood levels of thyroid hormone*
624 *that is considerably broader than the individual variance (Andersen, Pedersen, Bruun,*
625 *& Laurberg 2002). Therefore, in euthyroid individuals, serum T4 and T3 fluctuate*
626 *within a fairly narrow range (about 10% of the population variance), maintained by*
627 *the negative feedback relationship with serum TSH from the pituitary gland. This*
628 *normal variation creates a situation where single measures of free or total T4 and TSH*

are a somewhat imprecise measure of an individual's average T4 and TSH concentrations (Andersen et al. 2002)."

Several other areas of uncertainty are also highlighted by USEPA (2017). Specifically, USEPA (2017) noted that none of the five studies carried forward provided iodine intake levels (Section 6.5.3), which adds significant uncertainty to the estimates. Three of the 5 studies (Pop et al. 1999, 2003; Vermiglio et al. 2004) also have populations of less than 30 decreasing the statistical power of the studies (section 6.5.4) relied upon for establishing the relationship between changes in fT4 and neurodevelopmental changes. USEPA (2017) also noted uncertainties in regard to the analytical methods used to evaluate fT4 levels and while approaches are being introduced to standardize analytical methods, results at different time points and from different countries may vary considerably due to differences in analytical procedures (USEPA 2017). USEPA (2017) also notes that "there is uncertainty regarding the true fT4 levels at various percentiles in the distribution around the median output from the BBDR model. This is exemplified by the fact that in this analysis larger unit changes are being seen with increasing percentiles of fT4 in most analyses." Finally, other confounders such as iron deficiency were not considered in the analysis. Iron deficiency in pregnant mothers, which is noted in approximately 18% of pregnant women in the US (Cantor et al. 2015), may also be associated with hypothyroxinemia (Yu et al. 2015) and failing to directly account for a relationship between iron deficiency and hypothyroxinemia may introduce an uncertainty into this analysis.

While all these uncertainties are noted by USEPA (2017), there is no attempt to adjust the draft MCLG approach in any way to account for these uncertainties. Many of these, especially confounders such as iron deficiency in the study population and a lack of information on iodide intake, can have a significant effect in characterizing changes in thyroid hormone levels associated with changes in neurodevelopmental outcomes. In the absence of adequately accounting for these uncertainties, it is difficult to have confidence that BBDR model predictions of small changes in a specific thyroid hormone (e.g. fT4) may accurately predict the potential for neurodevelopmental effects.

The inadequacy of the USEPA (2017) literature review is substantiated by the comments of the External Peer Review for USEPA's Proposed Approaches to Inform the Derivation of a Maximum Contaminant Level Goal for Perchlorate in Drinking Water (Versar 2018). Comments regarding the USEPA (2017) literature search included:

- "The literature search produced ten studies (that assessed maternal serum FT4 concentrations as a continuous measure which did not observe an adverse effect on offspring neurocognition), as well as those in Group 2 that assessed serum FT4 as categorical measures. Although their inclusion may not necessarily be recommended in the final model, comparison of the estimated effects on the various neurocognitive outcomes with and without these may indeed inform the degree of uncertainty inherent in the present model. Several of the studies in Group 2 were able to demonstrate significant adverse outcomes (Berbel 2009 as one excellent example), and also their more global nature would help support the generalizability of the present model."
- "Excluding these studies lessens the power of the total sample size and thus the ability to detect an association between maternal hypothyroxinemia and any of the offspring outcomes, but provides what may be a somewhat exaggerated estimate of the potential adverse effects of perchlorate exposure. This approach is more conservative, to which there are pros and cons of doing so, toward derivation of a perchlorate MCLG. With this approach, the goal is to minimize exposure to the lowest perchlorate concentration associated with any number of adverse outcomes. I would favor the more liberal public health approach, which is inclusion of all available studies, whether they are positive or negative. Although the perchlorate MCLG may be higher, this latter approach would be consistent with using all available evidence to improve the scientific rigor of the proposed study question."

The peer reviewers also suggested a number of additional peer-reviewed studies that they felt should have been considered to inform BBDR modeling of the quantitative relationship between thyroid hormone levels and neurodevelopmental outcomes:

685 ∞ Bárez-López S, Jesus-Obregon M, Bernal J, Guadaño-Ferraz A. 2017. Thyroid Hormone
686 Economy in the Perinatal Mouse Brain: Implications for Cerebral Cortex Development.
687 Cerebral Cortex, 28(5): 1783-1793.
688 ∞ Bath S, Steer C, Golding J, Emmett P, Raymen M. 2013. Effect of inadequate iodine
689 status in UK pregnant women on cognitive outcomes in their children: results from the
690 Avon Longitudinal Study of Parents and Children (ALSPAC). The Lancet, 382(9889):
691 331-337.
692 ∞ Bernal J. 2017. Thyroid hormone regulated genes in cerebral cortex development.
693 Journal of Endocrinology, 232(2): R83-R97.
694 ∞ Casey B, Thom E. 2017. Subclinical Hypothyroidism or Hypothyroxinemia in
695 Pregnancy. The New England Journal of Medicine, 377(7): 701.
696 ∞ Casey B, Thom E, Peacemann A, Varner M, Sorokin Y, Hirtz D, Reddy U, Wapner R,
697 Thorp J, Saade G, Tita A, Rouse D, Sibai B, Iams J, Mercer B, Tolosa J, Caritis S,
698 VanDorsten JP. 2017. Treatment of Subclinical Hypothyroidism or Hypothyroxinemia in
699 Pregnancy. The New England Journal of Medicine, 376: 815-825.
700 ∞ Endendijk J, Wijnen H, Pop V, van Baar A. 2017. Maternal thyroid hormone
701 trajectories during pregnancy and child behavioral problems. Hormones and Behavior,
702 94: 84-92.
703 ∞ Hales C, Taylor P, Channon S, Paradice R, McEwan K, Zhang L, Gyedu M, Bakhsh O,
704 Muller I, Draman M, Gregory J, Dayan J, Rees D, Ludgate M. 2018. Controlled
705 Antenatal Thyroid Screening II: effect of treating maternal sub-optimal thyroid
706 function on childhood cognition. The Journal of Clinical Endocrinology and Metabolism,
707 103(4): 1583-1591.
708 ∞ Lazarus J, Bestwick J, Channon S, Paradice R, Maina A, Rees R, Chiusano E, John R,
709 Guaraldo V, George L, Perona M, Dall'Amico D, Parkes A, Joomun M, Wald NJ. 2012.
710 Antenatal Thyroid Screening and Childhood Cognitive Function. The New England
711 Journal of Medicine, 366: 493-501.
712 ∞ Taylor PN, Okosieme OE, Murphy R, Hales C, Chiusano E, Maina A, Joomun M,
713 Bestwick JP, Smyth P, Paradice R, Channon S, Braverman LE, Dayan CM, Lazarus JH,
714 Pearce EN. 2014. Maternal Perchlorate Levels in Women with Borderline Thyroid

Function During Pregnancy and the Cognitive Development of Their Offspring: Data from the Controlled Antenatal Thyroid Study. The Journal of Clinical Endocrinology & Metabolism, 99(11): 4291-4298.

In the draft MCLG approach, USEPA (2017) focused on five studies that evaluated the relationship of maternal fT4 and several neurodevelopmental endpoints (IQ, mental development index (MDI), psychomotor development index (PDI), standard deviation of reaction time), based on measurements of fT4 during early pregnancy. Results from previous studies have provided the basis for No Observed Effect Levels (NOELs) for health effects of perchlorate in the development of Reference Doses and currently recommended Health Reference Levels (HRLs), including Greer et al. (2002) in which adult men and women were exposed to perchlorate in drinking water at doses of 0.007, 0.02, 0.1, or 0.5 mg/kg/day for 14 days demonstrated a NOEL for perchlorate inhibition of radioiodide uptake by the thyroid NIS following exposure to 7 µg/kg/day. The point of departure from the Greer et al. (2002) study represents a perchlorate level that precedes the inhibition of iodine uptake by the thyroid. The NAS RfD developed based on the point of departure (POD) from this study is a deviation from the Agency's traditional approach of using a No Observed Adverse Effect Level (NOAEL) for regulatory actions. The NAS's use of a No Observed Effect Level (NOEL) is based on "using a nonadverse effect that is upstream of the adverse effect [which] is a more conservative and health protective approach".

While these studies have not been conducted in pregnant women (the population of interest for the draft MCLG approach), as noted by in USEPA (2017):

> *"…the BBDR model predicts very little difference in non-pregnant and first-trimester response to perchlorate. This likely occurs because the half-life of (organified) iodine in the adult thyroid is around six months, hence the availability of thyroidal iodine in the first trimester pregnant woman is determined to a very large extent by her nutrition and perchlorate exposure several years preceding pregnancy."*

This suggests that a comparison of the current modeling results to those from studies conducted in adults should provide insight into the predictions of the model and the conclusions regarding the changes in thyroid hormone levels that may result in neurodevelopmental effects.

742 The current draft approach for deriving the MCLG assumes any exposure to perchlorate reduces fT4 to
743 some extent (p. 3-17 of USEPA 2017). In addition, linear regression analyses conducted to evaluate
744 the relationship between changes in fT4 and neurodevelopmental effects further assumes any change
745 in fT4 results in some risk of neurodevelopmental effects. These assumptions are in contrast to the
746 results from Greer et al. (2002) in which exposures to perchlorate were as high as 500 µg/kg/day and
747 no impact on thyroid hormone levels was observed. This was true for both men and women. In
748 addition, in a study conducted by Braverman et al. (2006), 6 months of exposure to perchlorate in
749 capsules at doses up to 3 mg/day (approximately 40 µg/kg/day) was reported to have no effect on
750 thyroid function, including inhibition of thyroid iodide uptake as well as serum levels of thyroid
751 hormones, TSH, and Tg in a small group of volunteers.

752 USEPA (2017) notes (p. 6-16) that from results of the literature review, it appears the relationship
753 between maternal fT4 and fetal brain development has a temporal relationship, with this influence
754 likely being greatest in early pregnancy (i.e. prior to mid-gestation). The focus of the evaluation is on
755 gestational weeks 12, 13, and 16, where the mother's fT4 levels will have the greatest impact on the
756 fetus. This should allow for comparison to the model results in pregnant women to results from
757 previous studies focused on identification of perchlorate concentrations that would impact fT4 levels in
758 adult women, such as the Greer et al. (2002) study.

759 Based on the BBDR model predictions, USEPA (2017) estimates that a perchlorate dose of 0.3-0.4
760 µg/kg/day would result in a 1% increase in the proportion of the population with hypothyroxinemia
761 and a perchlorate dose of 2.1-2.2 µg/kg/day would result in a 5% increase in proportion of the
762 population with hypothyroxinemia. These modeling results suggest a potential for a significant change
763 in thyroid hormones, as well as adverse effects on neurodevelopment at doses of perchlorate exposure
764 for which there is evidence that decreases in fT4 are not observed. Based on the mode of action
765 proposed by USEPA (2017), decreases in fT4 and increases in TSH would be prerequisite steps for the
766 potential for neurodevelopmental effects. These changes in hormone levels are not observed in the
767 Greer et al. (2002) study following exposure up to 500 µg/kg/day. The draft MCLG approach suggests
768 population changes in fT4 would be observed that would shift the proportion of pregnant women that
769 would be hypothyroxinemic at doses of perchlorate below the previously defined NOEL (7 µg/kg/day).

Table 6 (Table 39 of USEPA 2017) provides the predicted dose of perchlorate per unit change in neurodevelopmental measure for low iodine intake individuals. Those for IQ are approximately at or above (6.5 – 45 µg/kg/day) the NOEL from Greer et al. (2002) and are associated with decreases in fT4 of 4.3 to 18.7%. The doses associated with other neurodevelopmental endpoints are 1.7 to 3.0 µg/kg/day and are associated with decreases in fT4 of 1.3 to 2.4%. These percent changes in fT4 are very small and considering the potential uncertainty and variability in measuring fT4 levels, there is a lack of evidence that such small changes in fT4 will result in clinical observations. Reference ranges for fT4 are 0.9 – 2.5 ng/dL in infants (0-5 days) and 0.9 – 1.7 ng/dL in adults (> 20 yrs) (https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8725). Thus, for an adult, at the low end of the reference range, we would expect a change from 0.900 to 0.878 ng/dL, a value that given the number of significant figures in the reference value would not be measurable. The dose of perchlorate estimated to result in a 1% or 5% increase in the proportion of hypothyroxinemic pregnant women is even lower, ranging from 0.3 to 2.2 µg/kg/day. USEPA (2017) findings are contrary to multiple studies in adults and pregnant women (Greer et al. 2002; Braverman et al. 2006; Téllez Téllez et al. 2005a, 2005b) provide robust evidence that no impact on iodine uptake or thyroid hormone levels would be expected at these dose levels. Based on the mode of action proposed by USEPA (2017), these precursor impacts are necessary to generate the neurodevelopmental effects derived from the BBDR model.

Table 6. Predicted Dose of Perchlorate per Unit Change in Neurodevelopmental Measure for Low Iodine Intake Individuals based on Central Effect Estimates at the Median fT4 level (USEPA 2017)

Study	Endpoint	ΔfT4 in pmol/L (% ΔfT4 from 0 dose perchlorate, iodine intake = 75 µg/day)	Dose of perchlorate per unit change in endpoint (µg/kg/day)[a]
Korevaar et al. (2016) Quadratic	IQ	-1.08 (12.2%)	23
Korevaar et al. (2016) USEPA Independent Analysis: Bivariate	IQ	-0.98 (11.1%)	20
Korevaar et al. (2016) USEPA Independent Analysis: Multivariate	IQ	-1.66 (18.7%)	45
Vermiglio et al. (2004)	IQ	-0.37 (4.3%)	6.5
Pop et al. (2003)	MDI	-0.15 (1.7%)	2.2
Pop et al. (2003)	PDI	-0.12 (1.3%)	1.7
Pop et al. (1999)	PDI	-0.12 (1.3%)	1.7
Finken et al. (2013)	SD of Reaction Time	-0.21 (2.4%)	3.0
BBDR model (USEPA 2017)	1% or 5% increase in proportion of hypothyroxinemic pregnant women[b]	1% or 5%	0.3 – 0.4[c] [1%] 2.1 – 2.2[c] [5%]

[a] Based on the regression analysis for the range of fT4 data within each study. Central beta estimates of the low iodide intake population (= 75 µg/day) are presented.

[b] Hypothyroxinemia defined as fT4 < 10th percentile

[c] Range based on gestational week used to perform the analysis (12 to 16 weeks).

788

DISCUSSION

A critical review of the (USEPA) 2017 report entitled "Draft Report: Proposed Approaches to Inform the Derivation of a Maximum Contaminant Level Goal for Perchlorate in Drinking Water", as well as the BBDR model that was proposed for use in derivation of the MCLG, was conducted. Overall, conducting this review and assessment of the BBDR model was beset by multiple challenges and the effort highlighted a number of uncertainties in the use of the model. The main challenges that the review presented were due to the complexity of the BBDR model itself. The co-authors of this review, who are widely considered to be experts in the area of PBPK and BBDR model development, found it difficult to evaluate the complex interactions of model parameters and their relationship to the predictions of the model. In our efforts to reproduce simulations provided in USEPA (2017), it was noted that instructions for running the model for different scenarios, and documentation of the rationale for the model parameter values associated with them, were sometimes inadequate; this deficiency, which is inevitable in a complex model, was exacerbated by the number of code scripts required to set the parameters used to generate the various results in the document. As a result, the ability to independently verify all aspects of the model were impeded by uncertainties associated with the steps necessary to reproduce figures and tables in the report, or to perform comparisons of model predictions to data for alternative exposure scenarios or studies.

As suggested by C.A.R. Hoare in his 1980 ACM Turing Award Lecture: "There are two ways of constructing a software design: One way is to make it so simple that there are obviously no deficiencies and the other way is to make it so complicated that there are no obvious deficiencies." By their nature, BBDR models are seldom simple; to the extent that BBDR models attempt to describe complex biological systems they will inherently be difficult to comprehend. The criticisms of the perchlorate PBPK model in this case study are not meant to suggest that the model is incorrect or un-useful, and they should not be taken as criticisms of the utility of BBDR modeling in general. Used appropriately, BBDR models can provide important information for better risk assessment decision-making. The issue that needs to be addressed in each case is whether a BBDR model is fit for the intended purpose of using it in the risk assessment.

The first use of PBPK modeling in risk assessments dates back to the 1980s (USEPA 1987) and yet the application of PBPK modeling to replace default dosimetry remains controversial, primarily due to

concerns regarding model uncertainty. To address these concerns, the OMB (2007) memorandum on risk analysis recommended the presentation of results from multiple dose-response approaches to provide a more robust risk characterization. In this scenario, a fit-for-purpose BBDR model can provide information on the most scientifically plausible risk estimate for comparison with the results of default approaches (Clewell et al. 2008). Consistent with this OMB recommendation, one focus of our evaluation was determining how the results of the BBDR modeling could inform the likelihood that the current perchlorate guideline (USEPA 2005), which is based on inhibition of thyroidal iodine uptake in adults, is also protective of concerns regarding neurodevelopmental effects of perchlorate. This question is discussed in the Conclusion.

The current BBDR model that was relied upon for the USEPA (2017) draft approach is an extension of previous models that have been validated and published in the peer-reviewed literature (Clewell et al. 2007; Merrill et al. 2003; Lumen et al. 2013). Similar values for key parameters have been successfully used across the previous models, yet changes were made in the current model or new parameters added (e.g. VCHNG, HCGreg, pTSH, KmNIS), often with little or no evidence or justification provided to support these revisions in the USEPA (2017) documentation. Additional support for these changes will be needed to provide validation of the current revisions to the BBDR model and to provide confidence in the predictions of changes in fT4 made by the model.

Certainly, confidence in the BBDR model predictions is undermined by the model's inability to simulate the results from the Steinmaus et al. (2016) study. In Appendix B of USEPA (2017), a comparison of the predicted changes in both fT4 and TSH from the BBDR model were compared to the results reported by Steinmaus et al. (2016) (reproduced in Figure 6). The Steinmaus et al. (2016) study was conducted to evaluate the potential for perchlorate exposure to impact thyroid hormone levels in pregnant women in San Diego. This comparison clearly highlights the differences between the model predictions and those from a published study. The baseline BBDR simulations with normal iodine intake (170 µg/day) demonstrate no change in fT4, which is consistent with other studies in which no impact on fT4 has been observed at doses up to 7 µg/kg/day (Greer et al. 2002; Braverman et al. 2006). The BBDR model underpredicted changes in fT4, even in the scenario with low dietary iodine intake (75 µg/day), when compared to the changes reported by Steinmaus et al. (2016). This discrepancy calls into question the ability of the model to predict changes in fT4 associated with

847 perchlorate exposure. In particular, the proposed MCLG approach depends on model predictions of
848 small changes in fT4 as low as approximately 1% (Table 6) being associated with unit changes in
849 neurodevelopmental endpoints. Predictions of this precision would require a level of model precision
850 that has not been demonstrated by comparisons to existing data.

851 Many of the changes in fT4 that are predicted by the draft MCLG approach to estimate impact on the
852 population distribution of fT4 and therefore result in per unit changes in neurodevelopmental
853 outcomes are small percent changes (some as low as a 1.3-4.3% change). This would appear to
854 suggest that the extended version of the BBDR model has a capability to estimate small changes in
855 fT4 with a level of precision that is not demonstrated by any adequate validation. In fact, BBDR model
856 predictions of fT4 underpredict observed data in human studies (Tables 1 and 3) by as much as 25-
857 35%. Moreover, considering the variability of fT4 in the populations of interest, there is uncertainty as
858 to whether these slight changes could be measured clinically, considering the greater impact of iodine
859 intake on hormone levels. Considering the lack of data to support critical parameters and
860 assumptions in the model, as well as the impact of the variability of iodine intake on model
861 predictions, it seems crucial that validation of the BBDR model by comparison with observed data be
862 used to provide confidence in the predictions of the BBDR model. However, the BBDR model clearly
863 fails the only comparison that has been conducted (Figure 6), with the BBDR model predictions falling
864 outside the bounds of the statistical confidence limits estimated for the Steinmaus et al. (2016)
865 relationship between perchlorate dose and fT4. Each of the components of the BBDR model combined
866 result in compounded uncertainty in the modeling results.

867 Until additional data are available to validate current extensions of the BBDR model to the pregnant
868 woman, the Greer et al. (2002) and Braverman et al. (2006) studies provide the critical information in
869 determining concentrations of perchlorate that do not result in significant inhibition of iodide uptake
870 and, therefore, impacts on fT4. Based on recommendations from the National Academy of Sciences
871 (2005), points of departure provided by these studies used in combination with uncertainty factors
872 were considered to be protective of sensitive subpopulations, this approach has previously been relied
873 upon to support guidelines for perchlorate in drinking water under the Safe Drinking Water Act (USEPA
874 2008), and has also been used more recently by JECFA (2011) and EFSA (2014) in their regulation of
875 perchlorate.

CONCLUSIONS

We applaud the USEPA for the application of a BBDR model in their draft MCLG approaches, as these models integrate the available science for a compound of interest. However, while the hormone component of the model is a scientific improvement in terms of incorporating the available biology, there is a lack of data to provide critical validation in multiple steps of the proposed approach and to support several assumptions/parameters within the BBDR model. In particular, while no major structural defects in the USEPA (2017) BBDR model were identified, there are a number of uncertainties in the model parameterization that call into question its use for predicting very small changes in clinical hormone values, such as a 1% change in fT4 (Tables 4-6) While the model prediction for 1% change in fT4 (0.3-0.4 µg/kg/day) would yield a POD lower than the USEPA (2005) RfD, that level of precision is a not supported by the comparison of the model predictions with available data. Nonetheless, the consistency of the model-predicted PODs based on the epidemiological endpoints (Table 6), and the relationship of these results with previous risk assessments based on biologically sound precursors (iodide inhibition in thyroid), indicate that the interim health standard would be sufficiently protective against the developmental neurological endpoints of concern, as illustrated in Figure 7, which compares the point of departure from the USEPA (2005) IRIS assessment with the PoDs calculated by the BBDR model in the USEPA (2017) report (Table 6). The USEPA (2005) RfD (red bar) is protective for all of the endpoints from epidemiological studies and is consistent with a change in population fT4 levels of less than 5%.

Beginning with the initial risk characterization for perchlorate (USEPA 2002), the fundamental underpinning of the agency's risk assessment approach has been the use of an obligatory precursor as a conservative basis for protecting against downstream health effects. As elaborated in the original documentation (USEPA 2003), the effects of perchlorate are mediated by the inhibition of thyroidal iodine uptake by perchlorate. Unless perchlorate concentrations in the blood are sufficient to disrupt iodine uptake, there is no plausible basis for suggesting an effect of perchlorate on thyroid hormone homeostasis or subsequent events leading to developmental or (in the rat) carcinogenic effects. The recent studies suggesting a relationship between perchlorate exposure and decreased fT4 do not impeach this causal relationship. Therefore, until the significant uncertainties in the current BBDR

model and draft MCLG approaches can be addressed, USEPA should continue to rely on the RfD approach based on inhibition of thyroidal iodine uptake (USEPA (2005), as recommended by the National Academy of Sciences (2005) for any further regulatory action. The USEPA (2005) RfD includes an intraspecies uncertainty factor of 10 "to protect the most sensitive population, the fetuses of pregnant women who might have hypothyroidism or iodide deficiency." None of the predictions of the BBDR model suggest that this uncertainty factor is inadequate.

FUNDING

This work was supported by the American Water Works Association (AWWA), an international, nonprofit, scientific and educational society dedicated to providing total water solutions assuring the effective management of water. Founded in 1881, the Association is the largest organization of water supply professionals in the world. No employee or representative of AWWA participated in the preparation of this article, or influenced its contents or interpretations, which are exclusively those of the authors.

REFERENCES

Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman WA, Laurberg P, Lazarus JH, Mandel SJ, Peeters RP, Sullivan S. 2017. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid, 27(3): 315-389.

Andersen S, Pedersen KM, Bruun NH, Laurberg P. 2002. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. The Journal of Clinical Endocrinology and Metabolism, 87(3): 1068-1072.

Berbel P, Mestre JL, Santamaría A, Palazón I, Franco A, Graells M, González-Torga A, Morreale de Escobar G. 2009. Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: the importance of early iodine supplementation. Thyroid, 19(5): 511-519. (As cited in USEPA 2017).

Berlin JA, Colditz GA. 1999. The role of meta-analysis in the regulatory process for foods, drugs, and devices. JAMA 281(9): 830-834 (As cited in NRC 2013).

Blount BC, Pirkle JL, Osterloh JD, Valentin-Blasini L, Caldwell KL. 2006. Urinary Perchlorate and Thyroid Hormone Levels in Adolescent and Adult Men and Women Living in the United States. Environmental Health Perspectives, 114(12): 1865-1871. (As cited in USEPA 2017).

Box GEP. 1976. Science and Statistics. Journal of the American Statistical Association, 71: 791–799.

Braverman L, Pearce EN, He X, Pino S, Seeley M, Beck B, Magnani B, Bleunt BC, Firek A. 2006. Effects of six months of daily low-dose perchlorate exposure on thyroid function in healthy volunteers. The Journal of Clinical Endocrinology and Metabolism, 91(7): 2721-2794.

Cantor AG, Bougatsos C, Dana T, Blazina I, McDonagh M. 2015. Evidence Summary: Other Supporting Document for Iron Deficiency Anemia in Pregnant Women: Screening and Supplementation. United States Preventive Services Task Force. Available at: https://www.uspreventiveservicestaskforce.org/Page/Document/evidence-summary22/iron-deficiency-anemia-in-pregnant-women-screening-and-supplementation.

944 Chevrier J, Harley KG, Kogut K, Holland N, Johnson C, Eskenazi B. 2011. Maternal thyroid function
945 during the second half of pregnancy and child neurodevelopment at 6, 12, 24, and 60 months of age.
946 Journal of Thyroid Research, 2011: 426427.

947 Clewell, H.J., Andersen, H.J., and Blaauboer, B.J. 2008. On the incorporation of chemical-specific
948 information in risk assessment. Toxicology Letters, 180:100-109.

949 Clewell RA, Merrill EA, Gearhart JM, Robinson PJ, Sterner TR, Mattie DR, Clewell HJ III. 2007.
950 Perchlorate and radioiodide across life stages in the human: using PBPK models to predict dosimetry
951 and thyroid inhibition and sensitive subpopulations based on developmental stage. Journal of
952 Toxicology and Environmental Health, Part A 70(5): 408-428.

953 Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ. 2003. Biologically
954 motivated computational modeling of formaldehyde carcinogenicity in the F344 rat. Toxicological
955 Sciences, 75: 432-447.

956 Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ. 2004. Human
957 respiratory tract cancer risks of inhaled formaldehyde: Dose-response predictions derived from
958 biologically-motivated computational modeling of a combined rodent and human dataset. Toxicological
959 Sciences, 82: 279-296.

960 de Escobar GM, Obregón MJ, del Rey FE. 2004. Maternal thyroid hormones early in pregnancy and
961 fetal brain development. [Review]. Best Practice and Research: Clinical Endocrinology and Metabolism,
962 18(2), 225-248. (As cited in USEPA 2017).

963 Delange FM. (Editor). 2000. Iodine deficiency (8th edition). Philadelphia, PA: Lippincott, Williams, and
964 Wilkins (As cited in USEPA 2017).

965 Dickersin K. 2002. Systematic reviews in epidemiology: Why are we so far behind? International
966 Journal of Epidemiology, 31(1): 6-12 (As cited in NRC 2013).

967 EFSA CONTAM Panel. 2014. Scientific opinion on the risks to public health related to the presence of
968 perchlorate in food, in particular fruits and vegetables: perchlorate in food, in particular fruits and
969 vegetables. EFSA Panel on Contaminants in the Food Chain. EFSA Journal, 12: 3869.

970 Finken MJJ, van Eijsden M, Loomans EM, Vrijkotte TGM, Rotteveel J. 2013. Maternal hypothyroxinemia
971 in early pregnancy predicts reduced performance in reaction time tests in 5- to 6-year-old offspring.
972 Journal of Clinical Endocrinology and Metabolism, 98(4): 1417-1426.

973 Fisher J, Lumen A, Latendresse J, Mattie D. 2012. Extrapolation of hypothalamic-pituitary-thyroid axis
974 perturbations and associated toxicity in rodents to humans: case study with perchlorate. Journal of
975 Environmental Science and Health. Part C, Environmental Carcinogenesis and Ecotoxicology Reviews,
976 30(1): 81-105.

977 Ghassabian A, Henrichs J, Tiemeier H. 2014. Impact of mild thyroid hormone deficiency in pregnancy
978 on cognitive function in children: lessons from the Generation R Study. Clinical Endocrinology and
979 Metabolism, 28(2): 221-232.

980 Glinoer D. 1997. The regulation of thyroid function in pregnancy: pathways of endocrine adaptation
981 from physiology to pathology. Endocrine Reviews, 18(3): 404-433 (As cited in USEPA 2017).

982 Greer MA, Goodman G, Pleus RC, Greer SE. 2002. Health effects assessment for environmental
983 perchlorate contamination: the dose response for inhibition of thyroidal radioiodine uptake in humans.
984 Environmental Health Perspectives, 110(9): 927-937.

985 Hoermann R, Broecker M, Grossmann M, Mann K, Derwahl M. 1994. Interaction of human chorionic
986 gonadotropin (hCG) and asialo-hCg with recombinant human thyrotropin receptor. The Journal of
987 Clinical Endocrinology and Metabolism, 78(4): 933-938.

988 Howdeshell KL. 2002. A model of the development of the brain as a construct of the thyroid system.
989 Environmental Health Perspectives, 110(Suppl 3): 337-348.

990 JECFA. 2011. Perchlorate. Geneva, Switzerland: WHO Food Additive Series: 63, FAO JECFA
991 Monographs 8, 72nd Meeting of Joint FAO/WHO Expert Committee on Food Additives (JECFA).

992 Korevaar TIM, Muetzel R, Medici M, Chaker L, Jaddoe VWV, de Rijke YB, Steegers EAP, Visser TJ,
993 White T, Tiemeier H, Peeters RP. 2016. Association of maternal thyroid function during early
994 pregnancy with offspring IQ and brain morphology in childhood: A population-based prospective
995 cohort study. The Lancet Diabetes and Endocrinology, 4(1): 35-43.

996 Korevaar, T. I., Steegers, E. A., de Rijke, Y. B., Schalekamp-Timmermans, S., Visser, W. E., Hofman,
997 A., Peeters, R. P. 2015. Reference ranges and determinants of total hCG levels during pregnancy: The
998 Generation R Study. European Journal of Epidemiology, 30(9), 1057-1066.

999 Li C, Shan Z, Mao J, Wang W, Xie X, Zhou W, Li C, Xu B, Bi L, Meng T, Du J, Zhang S, Gao Z, Zhang
1000 X, Yang L, Fan C, Teng W. 2014. Assessment of thyroid function during first-trimester pregnancy:
1001 What is the rational upper limit of serum TSH during the first trimester in Chinese pregnant women?
1002 Journal of Clinical Endocrinology and Metabolism, 99(1): 73-79. (As cited in USEPA 2017).

1003 Lumen A, George NI. 2017a. Evaluation of the risk of perchlorate exposure in a population of late-
1004 gestation pregnant women in the United States: Application of probabilistic biologically-based dose
1005 response modeling. Toxicology and Applied Pharmacology, 322: 9-14.

1006 Lumen A, George NI. 2017b. Estimation of iodine nutrition and thyroid function status in late-gestation
1007 pregnant women in the United States: Development and application of a population-based pregnancy
1008 model. Toxicology and Applied Pharmacology, 314: 24-38.

1009 Lumen A, Mattie DR, Fisher JW. 2013. Evaluation of perturbations in serum thyroid hormones during
1010 human pregnancy due to dietary iodide and perchlorate exposure using a biologically based dose-
1011 response model. Toxicol Sci 133: 320-341. doi: 10.1093/toxsci/kft078.

1012 Lumen A, McNally K, George N, Fisher JW, Loizou GD. 2015. Quantitative global sensitivity analysis of
1013 a biologically based dose-response pregnancy model for the thyroid endocrine system. Frontiers in
1014 Pharmacology, 6: 107.

1015 Männistö T, Surcel HM, Ruokonen A, Vääräsmäki M, Pouta A, Bloigu A, Järvelin MR, Hartikainen AL,
1016 Suvanto E. 2011. Early pregnancy reference intervals of thyroid hormone concentrations in a thyroid
1017 antibody-negative pregnant population. Thyroid, 21(3): 291-298 (As cited in USEPA 2017).

1018 McLanahan ED, Andersen ME, Campbell JL Jr, Fisher JW. 2009. Competitive inhibition of thyroidal
1019 uptake of dietary iodide by perchlorate does not describe perturbations in rat serum total T4 and TSH.
1020 Environmental Health Perspectives, 117: 731-738.

1021 McLanahan ED, Andersen ME, Fisher JW. 2008. A biologically based dose-response model for dietary
1022 iodide and the hypothalamic-pituitary-thyroid axis in the adult rat: Evaluation of iodide deficiency.
1023 Toxicological Sciences, 102: 241-253.

1024 Merrill EA, Clewell RA, Gearhart JM, Robinson PJ, Sterner TR, Yu KO, Mattie DR, Fisher JW. 2003. PBPK
1025 predictions of perchlorate distribution and its effect on thyroid uptake of radioiodide in the male rat.
1026 Toxicological Sciences, 73(2): 256-269.

1027 Modesto T, Tiemeier H, Peeters RP, Jaddoe VW, Hofman A, Verhulst FC, Ghassabian A. 2015. Maternal
1028 mild thyroid hormone insufficiency in early pregnancy and attention deficit/hyperactivity disorder
1029 symptoms in children. JAMA, 169(9): 838-845.

1030 Moleti M, Trimarchi F, Tortorella G, Candia Longo A, Giorgianni G, Sturniolo G, Alibrandi A, Vermiglio
1031 F. 2016. Effects of maternal iodine nutrition and thyroid status on cognitive development in offspring:
1032 A pilot study. Thyroid, 26(2): 296-305.

1033 Moncayo R, Zanon B, Heim K, Ortner K, Moncayo H. 2015. Thyroid function parameters in normal
1034 pregnancies in an iodine sufficient population. BBA Clinical, 3: 90-95. (As cited in USEPA 2017 –
1035 Figure A-33 as Moncayo et al. 2014).

1036 Morreale de Escobar G, Obregón MJ, Escobar del Ray F. 2000. Is Neuropsychological Development
1037 Related to Maternal Hypothyroidism or to Maternal Hypothyroxinemia? Journal of Clinical
1038 Endocrinology and Metabolism, 85(11): 3975-3987 (As cited in USEPA 2017).

1039 National Academy of Sciences. 2005. Health Implications of Perchlorate Ingestion. National Academy
1040 Press, Washington DC.

1041 Nicoloff JT, Low JC, Dussault JH, Fisher DA. 1972. Simultaneous measurement of thyroxine and
1042 triiodothyronine peripheral turnover kinetics in man. Journal of Clinical Investigation, 51(3): 473-483
1043 (As cited in USEPA 2017).

1044 Noten AM, Loomans EM, Vrijkotte TG, van de Ven PM, van Trotsenburg AS, Rotteveel J, van Eijsden M,
1045 Finken MJ. 2015. Maternal hypothyroxinaemia in early pregnancy and school performance in 5-year-
1046 old offspring. European Journal of Endocrinology, 173(5): 563-571.

1047 NRC. 2005. Health Implications of Perchlorate Ingestion. National Academies Press, Board on
1048 Environmental Studies and Toxicology. January, 2005.

1049 NRC. 2013. Critical Aspects of USEPA's IRIS Assessment of Inorganic Arsenic: Interim Report. National
1050 Research Council, Division on Earth and Life Studies, Board on Environmental Studies and Toxicology,
1051 Committee on Inorganic Arsenic. Available at:
1052 https://books.google.com/books?id=PflhCwAAQBAJ&lpg=PP1&dq=Critical%20Aspects%20of%20EPA's
1053 %20IRIS%20Assessment%20of%20Inorganic%20Arsenic%3A%20Interim%20Report&pg=PR10#v=o
1054 nepage&q=Critical%20Aspects%20of%20EPA's%20IRIS%20Assessment%20of%20Inorganic%20Arse
1055 nic:%20Interim%20Report&f=false.

1056 NRC. 2014. Review of USEPA's Integrated Risk Information System (IRIS) Process. National Research
1057 Council. Washington, DC: The National Academies Press. Available online:
1058 http://www.nap.edu/catalog/18764/review-of-epas-integrated-risk-information-system-iris-process

1059 NTP. 2015. Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for
1060 Systematic Review and Evidence Integration. National Toxicology Program, United States Department
1061 of Health and Human Services. Office of Health Assessment and Translation (OHAT), Division of the
1062 National Toxicology Program, National Institute of Environmental Health Sciences.

1063 OMB. 2007. Updated Principles for Risk Analysis. Office of Management and Budget. Available at:
1064 https://www.whitehouse.gov/sites/whitehouse.gov/files/omb/memoranda/2007/m07-24.pdf

1065 Oken E, Braverman L, Platek D, Mitchell ML, Lee SL, Pearce EN. 2009. Neonatal thyroxine, maternal
1066 thyroid function, and child cognition. The Journal of Clinical Endocrinology and Metabolism, 94(2):
1067 497-503.

1068 Panesar NS, Li CY, Rogers MS. 2001. Reference intervals for thyroid hormones in pregnant Chinese
1069 women. Annals of Clinical Biochemistry, 38(Pt 4), 329-332. (As cited in USEPA 2017).

1070 Pesce L, Kopp P. 2014. Iodide transport: implications for health and disease. International Journal of
1071 Pediatric Endocrinology, 2014(1): 8.

1072 Pop VJ, Kuijpens JL, van Baar AL, Verkerk G, van Son MM, de Vijlder JJ, Vulsma T, Wiersinga WM,
1073 Drexhage HA, Vader HL. 1999. Low maternal free thyroxine concentrations during early pregnancy are
1074 associated with impaired psychomotor development in infancy. Clinical Endocrinology, 50: 149-155.

1075 Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ. 2003. Maternal
1076 hypothyroxinemia during early pregnancy and subsequent child development: A 3-year follow-up
1077 study. Clinical Endocrinology, 59: 282-288.

1078 SAB. 2013. SAB Advice on Approaches to Derive a Maximum Contaminant Level Goal for Perchlorate.
1079 EPA-SAB-13-004. Washington, DC.

1080 Schlosser PM. 2016. Revision of the affinity constant for perchlorate binding to the sodium-iodide
1081 symporter based on in vitro and human in vivo data. Journal of Applied Toxicology, 36(12): 1531-
1082 1535 (As cited in USEPA 2017).

1083 Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, Nixon A, Pearce E, Soldin
1084 O, Sullivan S, Wiersinga W. 2011. Guidelines of the American Thyroid Association for the Diagnosis
1085 and Management of Thyroid Disease during Pregnancy and Postpartum. The American Thyroid
1086 Association Taskforce on Thyroid Disease during Pregnancy and postpartum. Thyroid, 21(10): 1081-
1087 1125.

1088 Steinmaus C, Pearl M, Kharrazi M, Blount BC, Miller MD, Pearce EN, Valentin-Blasini L, DeLorenze G,
1089 Hoofnagle AN, Liaw J. 2016. Thyroid hormones and moderate exposure to perchlorate during
1090 pregnancy in women in Southern California. Environmental Health Perspectives, 124(6): 861-867.

1091 Téllez Téllez R, Michaud Chacón P, Reyes Abarca C, Blount BC, Van Landingham CB, Crump KS, Gibbs
1092 JP. 2005a. Long-term environmental exposure to perchlorate through drinking water and thyroid
1093 function during pregnancy and the neonatal period. Thyroid, 15(9), 963-975.

1094 Téllez Téllez R, Michaud Chacón P, Reyes Abarca C, Blount BC, Van Landingham CB, Crump KS, Gibbs
1095 JP. 2005b. Chronic environmental exposure to perchlorate through drinking water and thyroid function
1096 during pregnancy and the neonatal period. Thyroid, 15(X).

1097 U.S. Environmental Protection Agency (USEPA). (1987). Update to the Health Assessment Document
1098 and Addendum for Dichloromethane (Methylene Chloride): Pharmacokinetics, Mechanism of Action,
1099 and Epidemiology. External Review Draft. EPA/600/8-87/030A. Office of Health and Environmental
1100 Assessment, USEPA, Washington, DC.

1101 USEPA. 2002. Perchlorate Environmental Contamination: Toxicological Review and Risk
1102 Characterization. External Review Draft. United States Environmental Protection Agency, Office of
1103 Research and Development. Washington, DC. NCEA-1-0503.

1104 USEPA. 2003. Draft Final Guidelines for Carcinogen Risk Assessment. United States Environmental
1105 Protection Agency, Risk Assessment Forum. Washington, DC. EPA/630/P-03/001A.

1106 USEPA. 2005. Integrated Risk Information System (IRIS) Chemical Assessment Summary: Perchlorate
1107 and Perchlorate Salts. United States Environmental Protection Agency, National Center for
1108 Environmental Assessment. Washington, DC.

1109 USEPA. 2008. Drinking Water: Preliminary Regulatory Determination on Perchlorate. United States
1110 Environmental Protection Agency. Federal Register 73(198): 60262-60282.

1111 USEPA. 2013. Advice on Approaches to Derive a Maximum Contaminant Level Goal for Perchlorate,
1112 Letter to The Honorable Bob Perciasepe, Acting Administrator, United States Environmental Protection
1113 Agency, Dated: May 29, 2013. EPA-SAB-13-004, 71 pages.

1114 USEPA. 2014a. Scoping and problem formulation for the identification of potential health hazards for
1115 the Integrated Risk Information System (IRIS) Toxicological Review of ethylbenzene [CASRN 100-41-
1116 4]. National Center Environmental Assessment, Office of Research and Development, United States
1117 Environmental Protection Agency, Washington, DC. EPA/635/R-14/198.

1118 USEPA. 2014b. Scoping and problem formulation for the identification of potential health hazards for
1119 the Integrated Risk Information System (IRIS) Toxicological Review of naphthalene [CASRN 91-20-3].
1120 National Center Environmental Assessment, Office of Research and Development, United States
1121 Environmental Protection Agency, Washington, DC. EPA/635/R-14/199.

1122 USEPA. 2017. Draft Report: Proposed approaches to inform the derivation of a maximum contaminant
1123 level for perchlorate in drinking water. Volume 1. Office of Ground Water and Drinking Water. United
1124 States Environmental Protection Agency. EPA-OGWDW. September 2017.

1125 Vermiglio F, Lo Presti VP, Moleti M, Sidoti M, Tortorella G, Scaffidi G, Castagna MG, Mattina F, Violi
1126 MA, Crisà A, Artemisia A, Trimarchi F. 2004. Attention deficit and hyperactivity disorders in the
1127 offspring of mothers exposed to mild-moderate iodine deficiency: A possible novel iodine deficiency
1128 disorder in developed countries. The Journal of Clinical Endocrinology and Metabolism, 89(12): 6054-
1129 6060.

1130 Versar. 2018. External Peer Review for USEPA's Proposed Approaches to Inform the Derivation of a
1131 Maximum Contaminant Level Goal for Perchlorate in Drinking Water. Report to USEPA Office of Ground
1132 Water and Drinking Water, March 2018.

1133 Yu X, Shan Z, Li C, Mao J, Wang W, Xie X, Liu A, Teng X, Zhou W, Li C, Xu B, Bi L, Meng T, Du J,
1134 Zhang S, Gao Z, Zhang X, Yang L, Fan C, Teng W. 2015. Iron deficiency, an independent risk factor
1135 for isolated hypothyroxinemia in pregnant and nonpregnant women of childbearing age in China. The
1136 Journal of Clinical Endocrinology and Metabolism, 100(4): 1594-1601.

1137 Zhang X, Yao B, Li C, Mao J, Wang W, Xie X, Teng X, Han C, Zhou W, Li C, Xu B, Bi L, Meng T, Du J,
1138 Zhang S, Gao Z, Yang L, Fan C, Teng W, Shan Z. 2016. Reference intervals of thyroid function during
1139 pregnancy: Self-sequential longitudinal study versus cross-sectional study. Thyroid, 26(12): 1786-
1140 1793 (As cited in USEPA 2017).

1141 Figure 1. Mode-of-action model for perchlorate toxicity proposed by the USEPA (2002).
1142 Inhibition of iodide uptake in the thyroid by perchlorate is an obligatory precursor for all
1143 downstream cancer and noncancer endpoints, including neurodevelopment.

1144 Figure 2: Structure of the Early Pregnancy BBDR (USEPA 2017)

1145 Figure 3: Variation in free T4 (fT4 in early pregnancy (as reported in USEPA 2017))

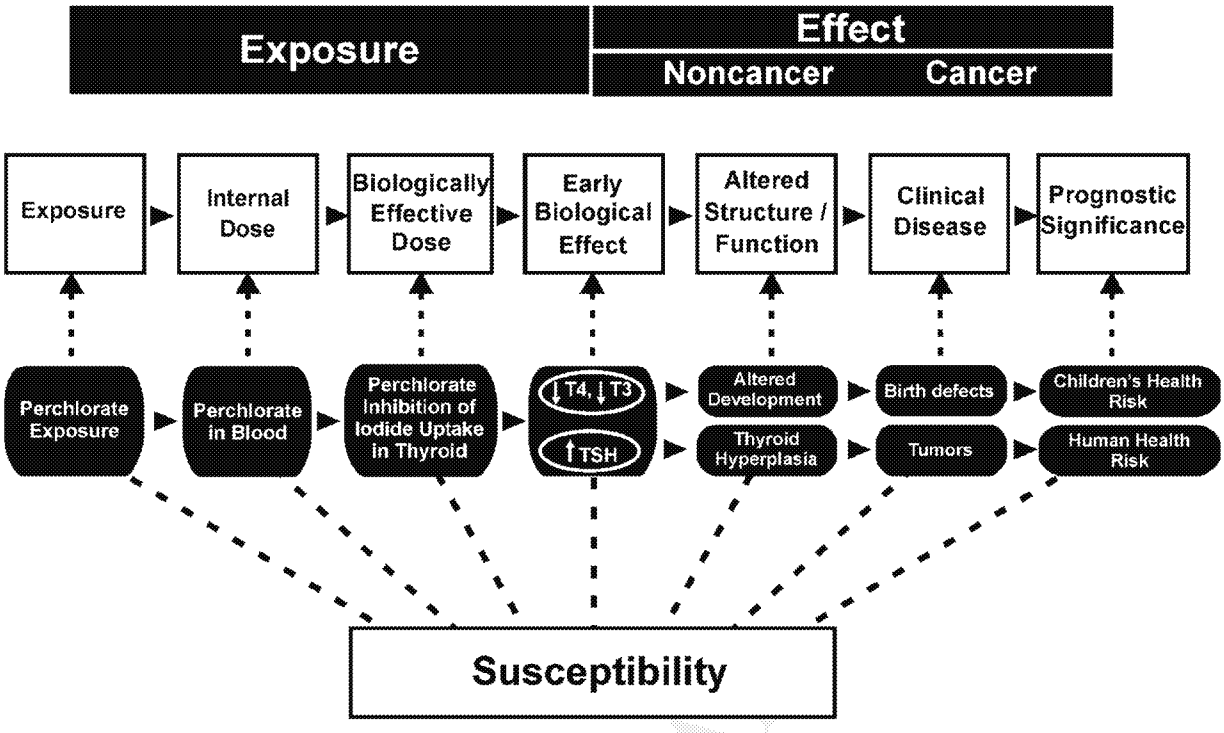
1146 Figure 4, Comparison of parameters controlling hCG-dependent changes in thyroidal uptake
1147 (VCHNG, green) and thyroid hormone production rate (HCGREG, purple) in the BBDR model as
1148 a function of gestational age. Despite the fact that both parameters are dependent upon hCG
1149 levels, the predicted trends across gestation are not consistent.

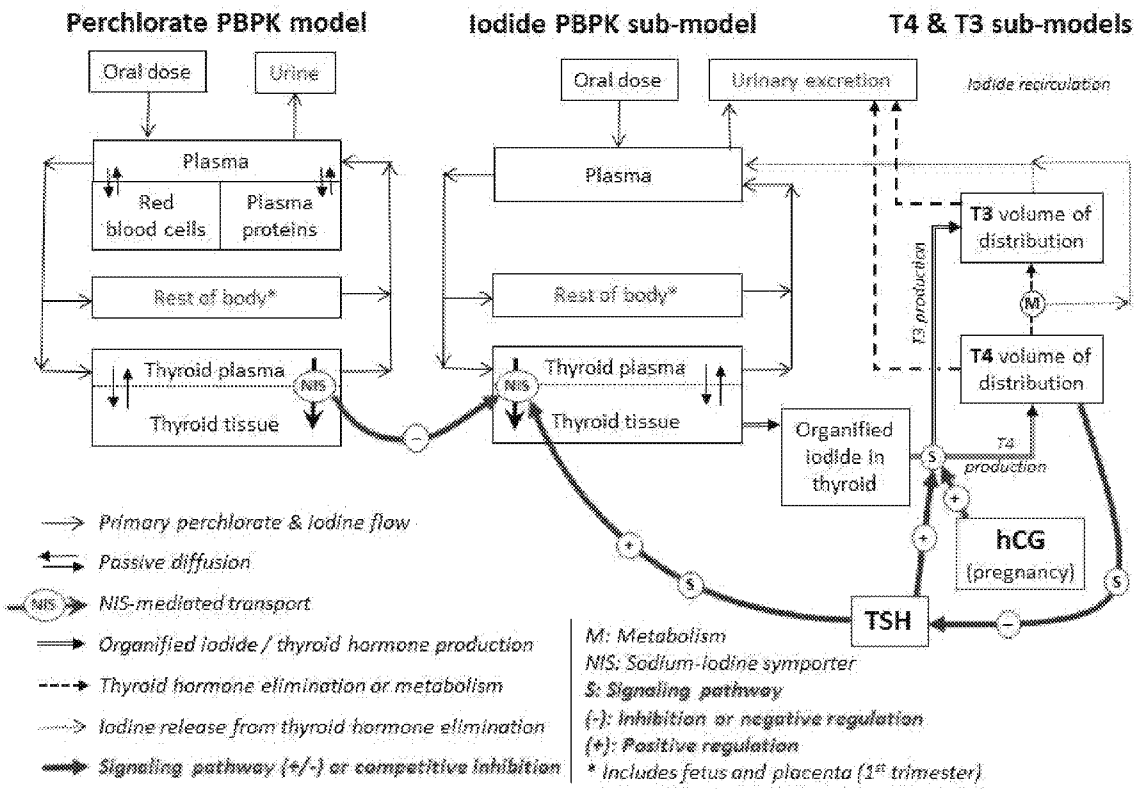
1150 Figure 5. Panel a: Model predictions for free T4 (fT4) in non-pregnant women as a function of
1151 iodine intake compared to data from NHANES 2007-2012 (USEPA 2017). Panel b: Underlying
1152 NHANES data without model predictions. Note the lack of evidence for any correlation
1153 between iodine intake and fT4 in the NHANES data in the range from 20 to 90 µg/d, in
1154 contrast to model predictions.

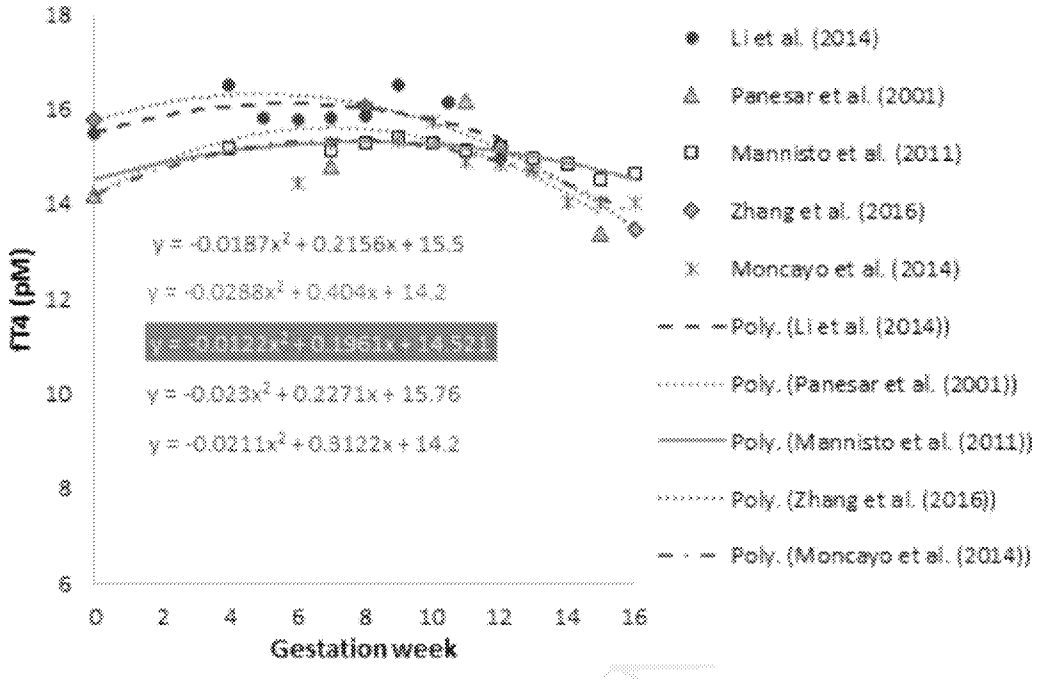
1155 Figure 6. Comparison of BBDR model predicted free T4 (fT4) changes as a function of
1156 perchlorate dose with data from Steinmaus et al. (2016). Reproduced from USEPA (2017).
1157 Blue boxes and diamonds represent the BBDR model predictions for median (170 µg/d) and
1158 low (90 µg/d) iodine intake populations (GW 13-16); red +'s represent the central estimate
1159 from the analysis of the Steinmaus et al. (2016) study and the red x's represent the upper
1160 and lower confidence limits for that estimate.

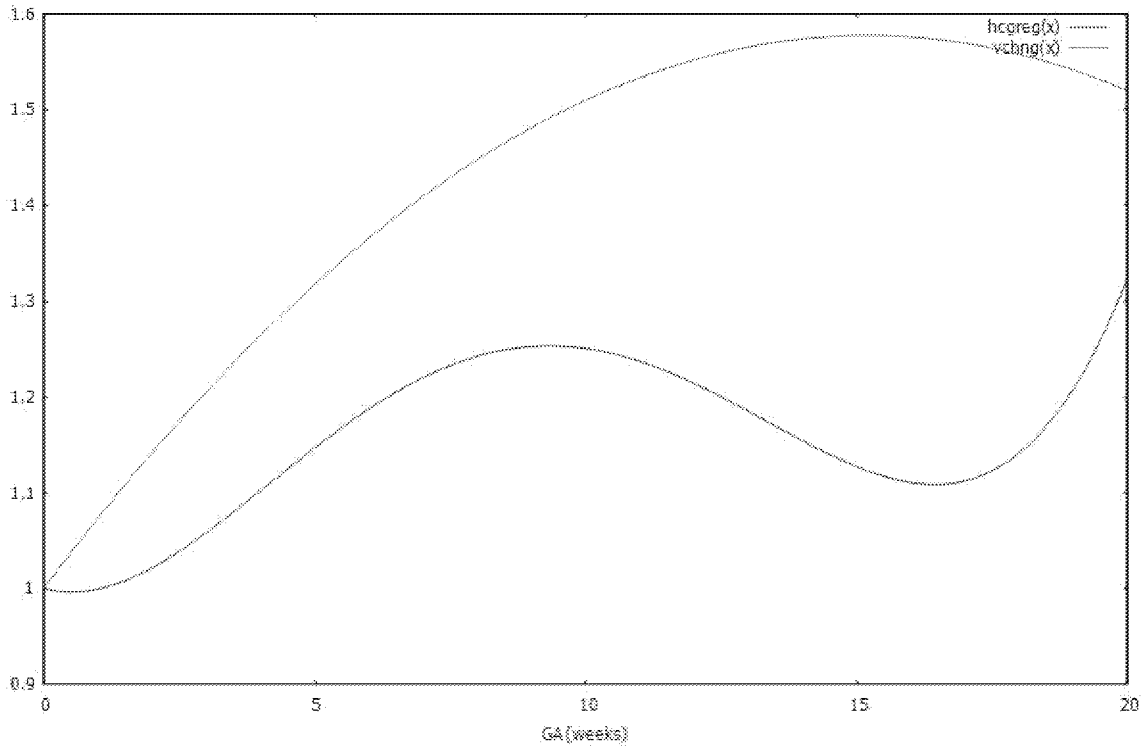
1161 Figure 7. Comparison of PoDs calculated using the USEPA (2017) BBDR model-based PoDs
1162 (blue and green bars) with the USEPA (2005) RfD (red bar).

1163

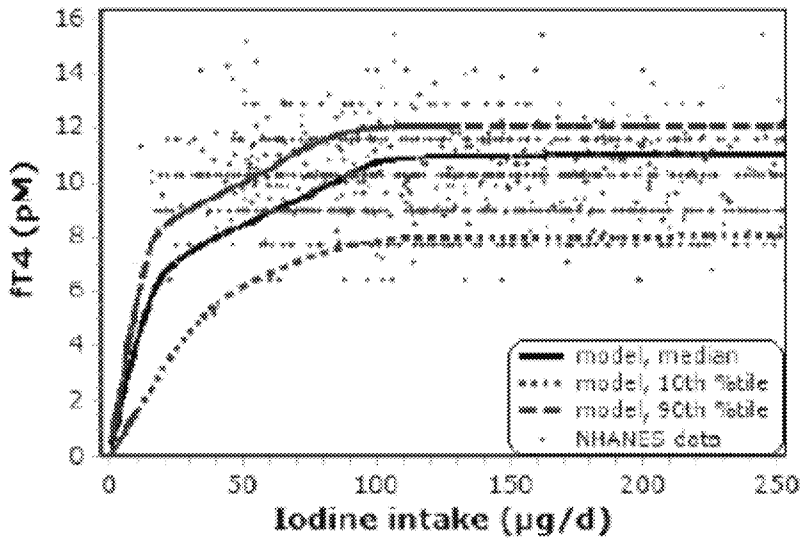




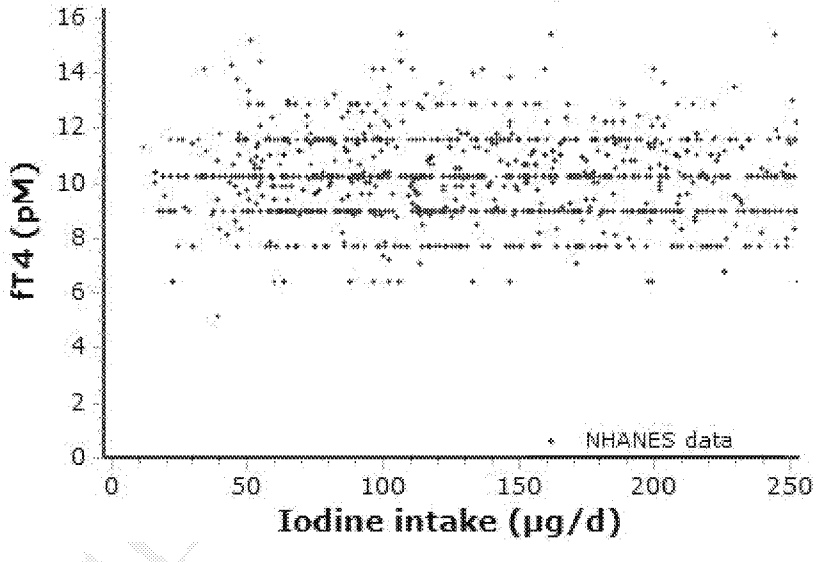


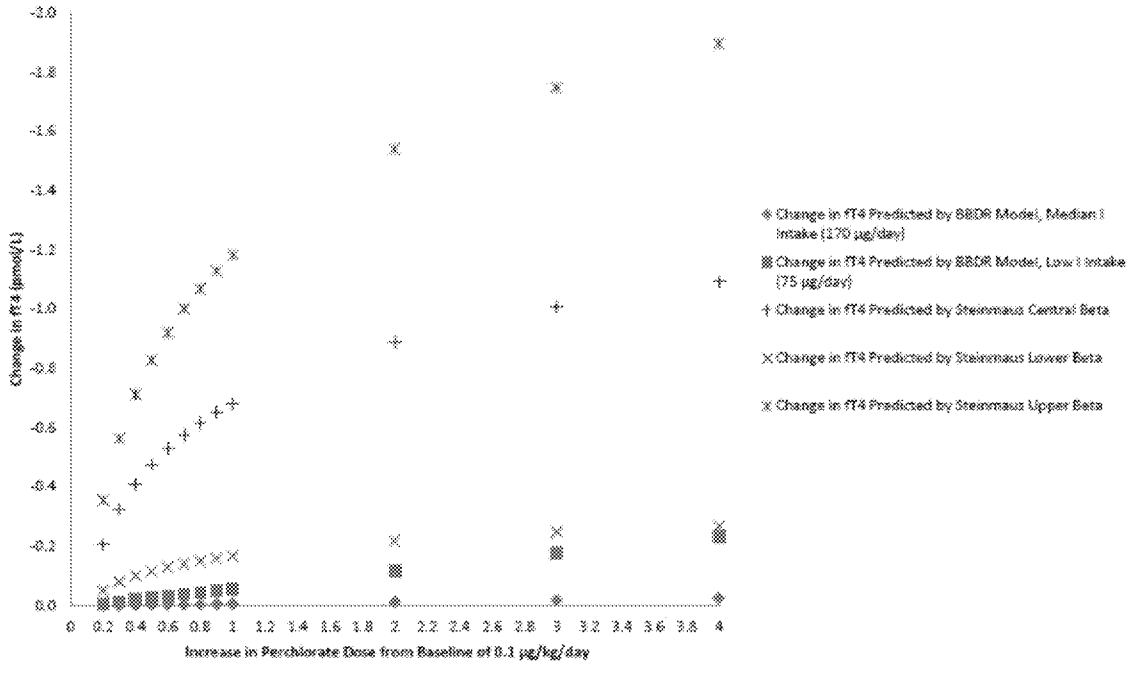


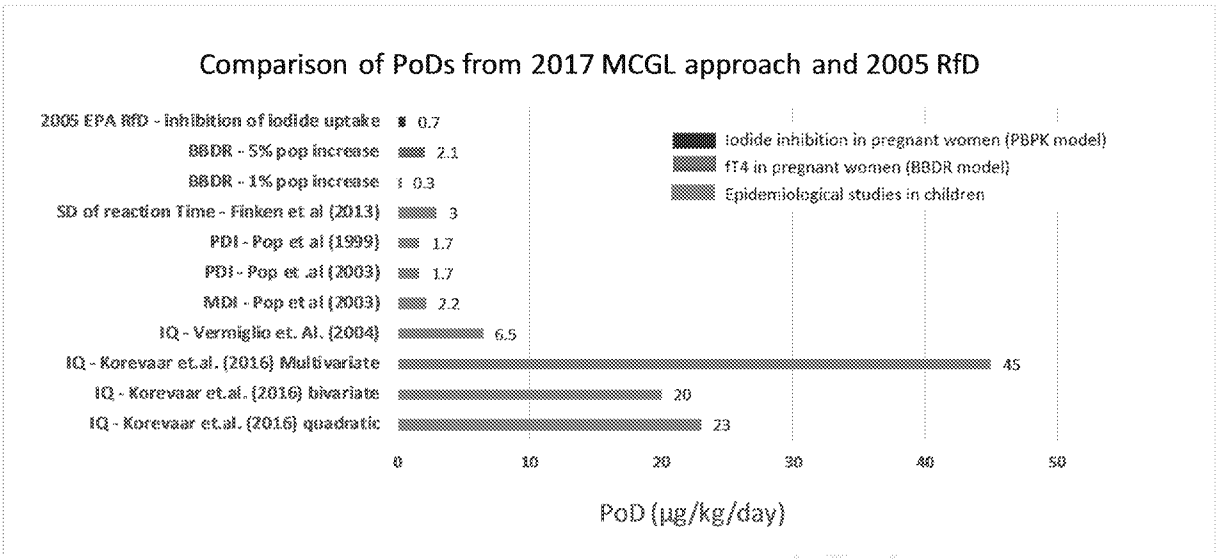
a.


fT4 (pM)
Iodine intake (µg/d)
model, median
model, 10th %tile
model, 90th %tile
NHANES data

b.


fT4 (pM)
Iodine intake (µg/d)
NHANES data





Highlights (maximum 125 characters, including spaces)

- The USEPA (2017) BBDR model plausibly describes perchlorate effects on thyroid hormone regulation during early pregnancy.
- The model is a valuable tool for investigating the effects of perchlorate on thyroid function during early gestation.
- BBDR modeling results indicate that the current USEPA RfD, based on adult effects, is also protective for fetal effects.
- However, current model uncertainties dictate against its use to replace the existing RfD for perchlorate.